

# **Modifying Post-Surgical Wound Healing**



A THESIS SUBMITTED IN FULFILMENT FOR  
DOCTOR OF PHILOSOPHY IN MEDICINE

by

**Rajan Sundaresan Vediappan**

MBBS, DLO, MS(ENT), MAOL

Department of Otorhinolaryngology Head and Neck Surgery

The Queen Elizabeth Hospital

Adelaide, Australia

January, 2021

## DEDICATED

---

*To the one who is seated at the right hand of the throne of the Majesty in heaven, and  
the one who lives in an unapproachable light, to Him be Glory and Honour.*

---

## DECLARATION

I, Rajan Sundaresan Vediappan certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Rajan Sundaresan Vediappan

## Table of Contents

|                                                                |           |
|----------------------------------------------------------------|-----------|
| <b>DECLARATION</b>                                             | <b>3</b>  |
| <b>TABLE OF CONTENTS</b>                                       | <b>4</b>  |
| <b>ACKNOWLEDGEMENT</b>                                         | <b>9</b>  |
| <b>PRESENTATIONS AND AWARDS</b>                                | <b>11</b> |
| <b>PUBLICATIONS ARISING FROM THIS THESIS</b>                   | <b>12</b> |
| <b>ABBREVIATIONS</b>                                           | <b>13</b> |
| <b>ABSTRACT</b>                                                | <b>18</b> |
| <b>LITERATURE REVIEW</b>                                       | <b>1</b>  |
| <b>1.INTRODUCTION</b>                                          | <b>1</b>  |
| <b>Wound Healing</b>                                           | <b>4</b>  |
| 1.1.1 Historical Perspective                                   | 4         |
| 1.1.2 Surgical trauma and wound healing:                       | 6         |
| 1.1.3 Wound Healing: Patho-physiology                          | 6         |
| 1.1.4 First Phase: <i>Haemostasis and</i> Inflammation:        | 7         |
| Platelets                                                      | 7         |
| Fibrin                                                         | 9         |
| Inflammation                                                   | 10        |
| 1.1.5 Second Phase: Proliferation                              | 10        |
| 1.1.6 Third Phase: Remodelling                                 | 11        |
| 1.1.7 Types of Wound Healing                                   | 13        |
| 1.1.8 Important factors that affect Wound Healing              | 13        |
| 1.1.9 Complications of wound Healing                           | 14        |
| 1.1.10 Adjuncts in Wound Healing                               | 15        |
| <b>Chitogel</b>                                                | <b>16</b> |
| 1.1.11 General Properties:                                     | 16        |
| 1.1.12 Pharmacokinetics and Toxicology                         | 18        |
| 1.1.13 Chitosan as a haemostatic agent:                        | 23        |
| 1.1.14 Chitosan as an anti-adhesiveagent:                      | 25        |
| 1.1.15 Chitosan as a weight-loss and/or anti-cholesterolagent: | 26        |

|        |                                                           |           |
|--------|-----------------------------------------------------------|-----------|
| 1.1.16 | Chitosan as a drug delivery agent:                        | 27        |
| 1.1.17 | Chitogel as a wound healing agent:                        | 28        |
| 1.1.18 | Chitogel as an anti-microbial /anti-biofilm agent:        | 29        |
|        | <b>Deferiprone (Def)</b>                                  | <b>30</b> |
|        | <b>Gallium Protoporphyrin</b>                             | <b>32</b> |
|        | <b>Combination of Def &amp; GaPP:</b>                     | <b>33</b> |
|        | <b>Drug release:</b>                                      | <b>35</b> |
|        | <b>Safety / Adverse Effects and Efficacy studies</b>      | <b>36</b> |
|        | <b>Manufacture /Chitogel Preparation:</b>                 | <b>39</b> |
|        | <b>2. WOUND HEALING IN CHRONIC RHINOSINUSITIS</b>         | <b>41</b> |
| 2.1.1  | Definition and Disease Burden Epidemiology:               | 41        |
| 2.1.2  | Epidemiology                                              | 42        |
| 2.1.3  | Patho-physiology                                          | 43        |
| 2.1.4  | Management of recalcitrant CRS                            | 46        |
| 2.1.5  | Medical Management                                        | 47        |
| 2.1.6  | Surgical Management                                       | 53        |
| 2.1.7  | Indications and aim of ESS                                | 54        |
| 2.1.8  | Outcomes of ESS                                           | 54        |
| 2.1.9  | Complications of ESS                                      | 55        |
| 2.1.10 | Sino nasal wound healing                                  | 56        |
| 2.1.11 | Adjuncts for improved wound healing in CRS                | 58        |
| 2.1.12 | Research in Nasal Packing:                                | 63        |
|        | <b>3. WOUND HEALING IN ABDOMINAL SURGERY</b>              | <b>70</b> |
|        | <b>Introduction</b>                                       | <b>70</b> |
| 3.1.1  | Anatomy & Physiology:                                     | 71        |
|        | <b>Post-Operative complications of Adhesion Formation</b> | <b>75</b> |
| 3.1.2  | Prevalence and consequences:                              | 75        |
|        | <b>Current options for adhesion prevention:</b>           | <b>78</b> |
| 3.1.3  | Surgical techniques to prevent adhesion:                  | 78        |
| 3.1.4  | Non-surgical Methods to prevent Adhesion                  | 79        |
| 3.1.5  | Research in abdominal adhesion prevention:                | 79        |
| 3.1.6  | Novel Anti – adhesion treatment:                          | 86        |
| 3.1.7  | Animal models for Abdominal Adhesion research:            | 87        |

|                                                                                                                                                                                           |            |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>4. WOUND HEALING IN SPINE SURGERY</b>                                                                                                                                                  | <b>90</b>  |
| Introduction:                                                                                                                                                                             | 90         |
| Pathophysiology of wound healing in the spinal canal:                                                                                                                                     | 91         |
| Symptomatology, Diagnosis and Management: Current                                                                                                                                         | 93         |
| strategies to prevent epidural fibrosis:                                                                                                                                                  | 94         |
| 4.1.1 Surgical methods:                                                                                                                                                                   | 95         |
| 4.1.2 Anti-inflammatory:                                                                                                                                                                  | 98         |
| 4.1.3 Barrier method:                                                                                                                                                                     | 99         |
| <b>5. SUMMARY OF LITERATURE REVIEW AND RESEARCH QUESTION:</b>                                                                                                                             | <b>103</b> |
| <b>6. CHITOGEL WITH DEF - GAPP IN ENT-ENDOSCOPIC SINUS SURGERY</b>                                                                                                                        | <b>104</b> |
| 6.1.1 Aim and Scope of Study                                                                                                                                                              | 106        |
| 6.1.2 Chitogel™ improves post-operative wound healing and patient outcomes in recalcitrant Sinusitis after ESS: Adding Deferiprone and Gallium Protoporphyrin, does it make a difference? | 107        |
| <b>7. CHITOGEL WITH DEF - GAPP IN ABDOMINAL SURGERY</b>                                                                                                                                   | <b>136</b> |
| 7.1.1 A Novel Rat Model to Test Intra-Abdominal Anti-Adhesive Therapy                                                                                                                     | 138        |
| 7.1.2 Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a Rat Model                                                       | 159        |
| <b>8. CHITOGEL WITH DEF IN SPINE SURGERY</b>                                                                                                                                              | <b>194</b> |
| 8.1.1 Paper 4: Prevention of peridural adhesions in spinal surgery: Assessing safety and efficacy of Chitogel with Deferiprone in a Sheep model                                           | 195        |
| <b>9. SYNOPSIS OF THESIS</b>                                                                                                                                                              | <b>223</b> |
| Summary of Thesis:                                                                                                                                                                        | 223        |
| Chitogel with Def-GaPP in ENT-Endoscopic Sinus Surgery (ESS):                                                                                                                             | 224        |
| Chitogel with Def-GaPP in Abdominal Surgery:                                                                                                                                              | 225        |
| Chitogel with Def in Spine Surgery:                                                                                                                                                       | 227        |
| CONCLUSION:                                                                                                                                                                               | 228        |
| <b>10. APPENDIX</b>                                                                                                                                                                       | <b>230</b> |
| Appendix 1: Flow chart of Project 1 Clinical trial                                                                                                                                        | 230        |
| Appendix 2: Consent and proforma                                                                                                                                                          | 231        |

|                                                              |            |
|--------------------------------------------------------------|------------|
| <b>Appendix 3: Patient reported Objective score sheet</b>    | <b>243</b> |
| <b>Appendix 4: Rat abdominal study Clinical record sheet</b> | <b>247</b> |
| <b>11. BIBLIOGRAPHY:</b>                                     | <b>248</b> |





## ACKNOWLEDGEMENT

What do I have that I have not received ? Everything that I have is a gift and I sincerely acknowledge the contribution of my parents, teachers, friends and colleagues who have made this journey so beautiful and full of joy.

At the outset I place on record the immense inspiration and influence that my supervisors have had on this part of my career. Prof. Peter John Wormald's drive and zeal for scientific and human endeavour has challenged and enabled me to come to Adelaide. Taking up this PhD programme under his able guidance was a blessing. His leadership in Adelaide and around the world in the field of ENT surgery is phenomenal and this PhD will enable us in our dream to emulate that in some fraction across the globe in India. Prof/A Alkis Psaltis is a great teacher, mentor and friend at the TQEH and he has never hesitated to teach and guide. Prof/A Sarah Vreugde cannot be thanked in few words, but suffice to say, 'she is a driving force for all the scientific research that was undertaken'. Dr Sarah's open door policy enabled me to walk up to her office and clarify doubts and her intense editorial skills are evident in my publications being accepted.

Research work at The Queen Elizabeth Hospital, Basil Hetzel Institute and The Memorial Hospital wouldn't have been so enjoyable if not for colleagues, researchers, nursing staff and overseas surgeons visiting and learning from each other. I sincerely thank the ENT team: Dr's Steve Floreani, Rowan Valentine, Neil Tan, John Scott, Yvonne C, Yazeed S, Vikram P, Jae Murphy, Satish Paramasivam, Giri Krishnan, Rachel Goggin, Stephen Kao, Michael, Anna Megow, Jate L, all have taught me at least one thing to be a better surgeon and a scientist. Ahmed Bassiouni needs a special mention because he is one of his kind, great friend and colleague who made calculations simple and straight.

Lyn Martin and Annette were not only efficient secretaries to the supervisors but also lovely facilitators to us researchers and visiting faculty at the TQEH. Catherine Bennett and Clare Cooksey's contributions at various stages of the research projects were crucial and their patient managerial skills made managing the ethics committee and research at the labs an easy one. Kathryn Hudson, Rebecca Anderson, Gwenda Graves and HDR PG co-ordinator Prue Cowled at the BHI were amazing colleagues and friends for life, they along with various other research group members they made work at BHI an easy sail. All these would not have been a reality but for the support and encouragement to take a 'step of faith' in travelling to Adelaide on study leave by Prof Dr Regi Thomas, my Senior colleague, friend, mentor and brother in Christ, and my Institution Christian Medical College, Vellore for giving me an opportunity to explore and expand my quest for knowledge and passion to serve. And also, The Hospital Research foundation and The University of Adelaide for putting their trust and supporting me with Australian Government Research Training Program Scholarship.

My family has been a bed rock of trust and support, my father Late John Sundaresan V always said "your goal in life is to first and foremost to study, second was to study and third was also study. These words have stuck to my heart and have enabled me to take up challenges and do what I could, and I hope that he would be watching from Heaven and be proud of this achievement too. Our mother Lydia Kanthimathi, who sacrificially allowed me to go on this journey when she needed me the most, and my siblings Sukumaran and Supriya for their genuine love and affection. Last, but most importantly my better self, Dr Beula Subashini a determined woman, who ran tirelessly towards her passion and carried me along. To our boys Ashish and Anugrah who have made this journey a pleasure and memorable part of our lives.

To God be the Glory.

## PRESENTATIONS and AWARDS ARISING FROM THIS THESIS

1. The 28th TQEH Research Expo, at the Basil-Hetzel-Institute, Adelaide ,10th October 2019: Oral presentation

PUBLICATIONS ARISING FROM THIS THESIS

1. Vediappan, R. S., Bennett, C., Bassiouni, A., Smith, M., Finnie, J., Trochsler, M., Psaltis, A. J., Vreugde, S., & Wormald, P. J.. A Novel RatModel to Test Intra-Abdominal Anti-adhesiveTherapy. *Frontiers in surgery*, 7, 12.  
<https://doi.org/10.3389/fsurg.2020.00012>  
Submitted to *Visceral Surgery*, a section of the Journal *Frontiers of Surgery*  
16.05.2019 and accepted: 06 March 2020 Published online: 08 April 2020
2. Vediappan RS, Mascarenhas A, Nguyen-Hoang A, AnneFong S, Jukes A, Richter K, Bassiouni A, Patel S, Chryssidis S, Otto S, Moratti S, James Psaltis A, Vreugde S, Wormald PJ. Prevention of peridural adhesions in spinal surgery: Assessing safety and efficacy of Chitogel with Deferiprone in a sheepmodel. *J ClinNeurosci*. 2020 Feb;72: 378-385.doi: 10.1016/j.jocn.2019.12.042.31948884. Submitted 6 September 2019, Accepted 16 December 2019
3. Rajan Sundaresan Vediappan, Catherine Bennett, Clare Cooksley, John Finnie, Markus Trochsler, Ryan D Quarrington, Claire F Jones, Ahmed Bassiouni, Stephen Moratti, Alkis J Psaltis, Guy Maddern, Sarah Vreugde, Wormald PJ Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a RatModel, *PLOS ONE*, Submitted on 15 May 2020, Accepted for publication on 26 December 2020
4. Rajan Sundaresan Vediappan, Catherine Bennett, Clare Cooksley, Ahmed Bassiouni, John R Scott, Yazeed Sulaiman, Jate Lumyongsatien, Stephen Moratti, Alkis J Psaltis, Sarah Vreugde, Wormald PJ Chitogel™ improves post-operativewoundhealing and patientoutcomes in recalcitrant Sinusitis after Endoscopic Sinus Surgery?  
Submitted for publication and Paperisunderreviewwith the journal  
- The Laryngoscope.

## ABBREVIATIONS

|        |                                                 |
|--------|-------------------------------------------------|
| ADP    | Adenosine diphosphate                           |
| ADSS   | Adelaide Disease Severity Score                 |
| BALB/c | Binaural Alternate Loudness Balance             |
| BCE    | Before the Common Era                           |
| CD     | Chitosan-Dextran                                |
| CMC    | Carboxy Methyl cellulose                        |
| CRS    | Chronic rhinosinusitis                          |
| CRSsNP | Chronic rhinosinusitis without nasal polyps     |
| CRSwNP | Chronic rhinosinusitis with nasal polyps        |
| Def    | Deferiprone                                     |
| ECM    | Extra cellular matrix                           |
| EGF    | Epidermal growth factor                         |
| EPS    | Extracellular polymeric substances              |
| ESS    | Endoscopic sinus surgery                        |
| FBSS   | Failed back surgery syndrome                    |
| FDA    | Food and Drug Administration                    |
| FE-PCF | Full-Endoscopic Posterior Cervical Foraminotomy |
| FESS   | Functional endoscopic Sinus Surgery             |
| GaPP   | Gallium Protoporphyrin                          |
| HPA    | Hypothalamus-pituitary-adrenal                  |
| HTS    | Hypertrophic scar                               |
| IAI    | Intra-abdominal infection                       |
| LAPAD  | LAParotomy or LAParoscopy and Adhesiolysis      |
| LBA    | Lower back ache                                 |
| LKS    | Lund-Kennedy Score                              |

|              |                                                    |
|--------------|----------------------------------------------------|
| LPS          | Lipopolysaccharide                                 |
| Mabs         | Monoclonal antibodies                              |
| MEF          | Micro-endoscopic foraminotomy                      |
| MIST         | Minimally invasive sinus technique                 |
| MLKS         | Modified Lund-Kennedy Score                        |
| MRI          | Magnetic resonance imaging                         |
| MRSA         | Methicillin resistant <i>Staphylococcus aureus</i> |
| NELA         | National Emergency Laparotomy Audit                |
| QOL          | Quality of life                                    |
| RCT          | Randomized control trials                          |
| ROS          | Reactive oxygen species                            |
| SCAR         | Surgical and Clinical Adhesions Research           |
| SEB          | <i>S. aureus</i> enterotoxin                       |
| SNOT – 22    | Sino-Nasal Outcome Test 22                         |
| TGF- $\beta$ | Transforming growth factor                         |
| TNF          | Tumour Necrosis Factor                             |
| TSST         | Toxic shock syndrome toxin                         |
| VAS          | Visual analogue scale                              |
| vWF          | Von Willebrand factor                              |

## Figure Legends:

|                                                                                                                                                                                                                                                                                       |            |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>Figure 1: Schematic representation of the possibilities of processing chitosan into different forms for medical use.....</b>                                                                                                                                                       | <b>3</b>   |
| <b>Figure 2: Steps of Haemostasis; Intrinsic and Extrinsic Pathways Adapted from (13) with permission. ....</b>                                                                                                                                                                       | <b>7</b>   |
| <b>Figure 3: Activation of Platelets in clot formation, adapted with permission from (13)...</b>                                                                                                                                                                                      | <b>8</b>   |
| <b>Figure 4: Figure 4 Adapted from Scott-Brown et.al representing the various phases of wound healing and cellular recruitment (29) .....</b>                                                                                                                                         | <b>12</b>  |
| <b>Figure 5: The chemical structure of N-succinyl-chitosan (12).....</b>                                                                                                                                                                                                              | <b>18</b>  |
| <b>Figure 6: XR and NIR-fluorescence imaging of mice injected with Chitogel(42).....</b>                                                                                                                                                                                              | <b>20</b>  |
| <b>Figure 7: Images of H&amp;E stain of surrounding tissues at the Chitogel subcutaneous injection site at (A) 4 hours and (B) 24 hours post injection in mice. *Indicates Chitogel(43) .....</b>                                                                                     | <b>21</b>  |
| <b>Figure 8: Determination of drug release kinetics.....</b>                                                                                                                                                                                                                          | <b>36</b>  |
| <b>Figure 9: Plasma concentration (<math>\mu\text{g/ml}</math>) <math>\pm</math> standard deviation of deferiprone (Def) over 6 days, n=4. The maximum Def concentration was reached after 1 day (0.85 <math>\mu\text{g/ml}</math> Def) in the 4 sheep treated with Def-GaPP.....</b> | <b>38</b>  |
| <b>Figure 10A: Example of a HPLC chromatogram of plasma samples. No gallium-protoporphyrin<sup>89</sup> was detected for any of the 4 sheep treated with Def-GaPP.....</b>                                                                                                            | <b>38</b>  |
| <b>Figure 11: A. Depicting the multiple layers of the Intestinal walls, B. Peritoneum – electron microscopy depicting mesothelial cells with microvilli, with permission(23)....</b>                                                                                                  | <b>72</b>  |
| <b>Figure 12: Illustration of spinal canal wound healing leading to adhesion formation<sup>387</sup> ..</b>                                                                                                                                                                           | <b>93</b>  |
| <b>Figure 13: Illustration depicting the various treatment strategy to prevent spinal adhesion, adapted with permission 387 .....</b>                                                                                                                                                 | <b>94</b>  |
| <b>Figure 14: Illustrative diagram showing the A. Cervical disc prolapse on an MRI, I - Sagittal view and II - Axial view, B. Location of pathology in relation to the lamina of the cervical vertebrae posteriorly, C. Placement of endoscope and excision of the compres .....</b>  | <b>97</b>  |
| <b>Figure 15: Illustration of laminectomy on sheep exposing the dura mater (White arrow) and MRI sagittal view after treatment (red arrow).....</b>                                                                                                                                   | <b>102</b> |

**Tables:**

|                                                                                              |           |
|----------------------------------------------------------------------------------------------|-----------|
| <b>Table 1: Platelet alpha granule components and their role in wound healing (14) .....</b> | <b>9</b>  |
| <b>Table 2: adapted from (30) Historical aspects of topical wound agents .....</b>           | <b>15</b> |
| <b>Table 3: Animal toxicology studies evaluating chitosan.....</b>                           | <b>22</b> |
| <b>Table 4:Animal toxicology studies evaluating chitosan (continued) .....</b>               | <b>23</b> |
| <b>Table 5: Various anti-adhesion agents researched .....</b>                                | <b>80</b> |
| <b>Table 6: Various treatment options researched .....</b>                                   | <b>98</b> |





## ABSTRACT

“Surgery is a profession defined by its authority to cure by means of bodily invasion. The brutality and risks of opening a living person's body have long been apparent, the benefits only slowly and haltingly worked out”, says Atul Rawande on reviewing 200 yrs. of Surgery as a specialty in NEJM. My research focuses on working out these benefits, specifically looking at reduction of scar tissue formation in ENT, Abdominal & Spine surgery.

Scar tissue formation is an outcome of healing process that can be excessive due to inflammation or infection and thereby has the ability to curtail the benefits or warrant revision surgery. Multiple strategies have been tested and employed thus far and none have given favourable results without causing additional harm or economic burden in health care costs. I propose to use a hydrogel synthesized by combining Chitosan and Dextran aldehyde - Chitin is an exoskeleton extracted polymer and Dextran Aldehyde a sugar, with added novel drugs Deferiprone and Gallium Protoporphyrin providing additional anti scarring and antibiotic properties which could potentially augment the healing properties of the gel.

I have conducted 3 types of studies. There are 2 animal studies and a Phase 1 Human clinical trial. The animal studies are an abdominal surgery rat model and a spine surgery sheep model. These studies show the safety and efficacy of this chitogel-drug combination at various dosages and illustrate the healing benefits of gel-drug combination.

.

# LITERATURE REVIEW

## 2. INTRODUCTION

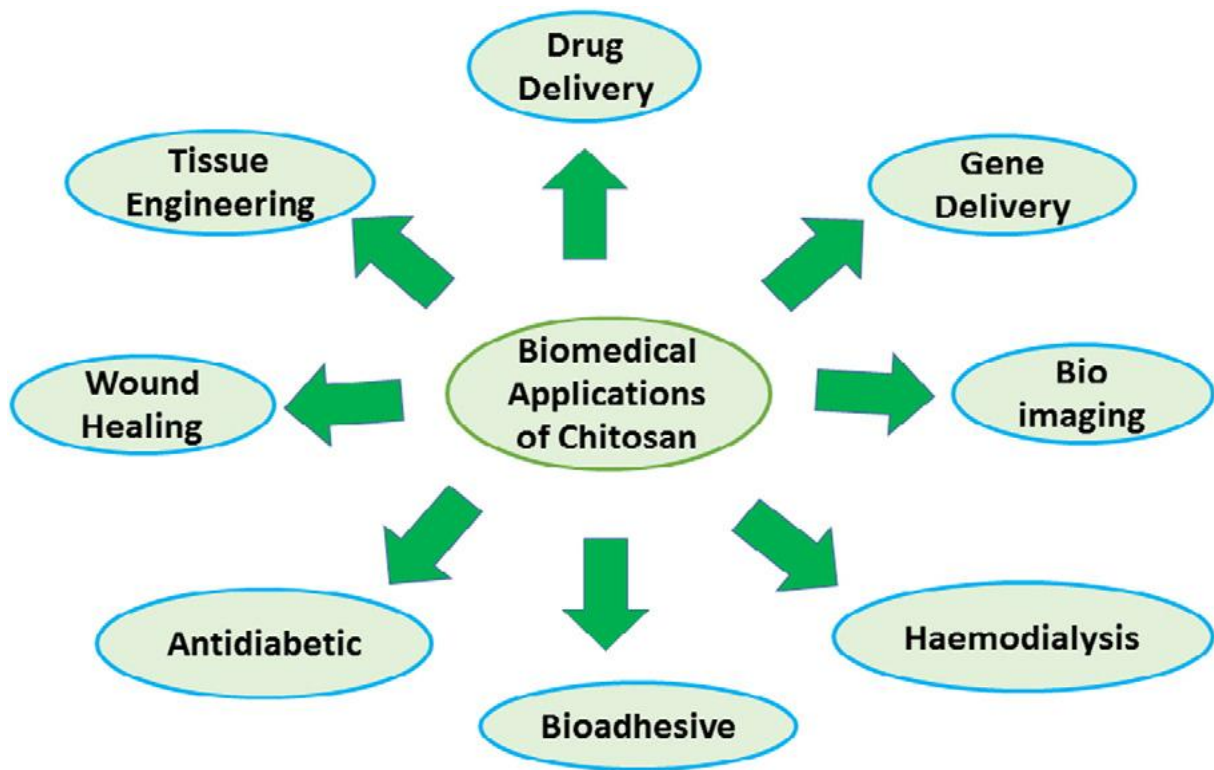
Surgery is a 'risky art' turned scientific discipline, wherein disease conditions were treated by creating bodily wound, these wounds have had 3 principles challenges right from its inception, namely bleeding, pain and infection. Not all wound healing is in-conspicuous and un-noticeable, some remain as scars, and these scars may hide disorderly healing. Wound healing and orderly restoration of broken tissues were human needs since the beginning of the time, 'I will restore you to health and heal your wounds,' (Jeremiah 30:17 KJV)<sup>1</sup> is hence the promise of God in the Bible.

Practitioners of medicine have been studying wound healing over the ages and there has been constant attempt to improve outcomes of the healing process. Attempts have been made to regulate the petite steps of scar formation by the ways the wound is created, the way it is put back together and even by the materials it is being held together by. Susrutha & Charaka - ancient Indian surgeons have dedicated 2 chapters in their treatise *Suśrutasamhitā* in the 1<sup>st</sup> Millennium Before the Common Era (BCE), to the management of wounds caused in battle and in domestic life. They have laid out a detailed description of wound creation and also describe how to treat poorly healing wounds<sup>2</sup> using surgical techniques and medicinal herbs. The aim of this thesis is to explore scar tissue formation in different surgical specialties and evaluate the wound healing effects of Chitogel-Drug combination.

Scar tissue is connective tissue disease referred to collectively as 'fibrosis'. This is generally excessive formation of connective tissue during wound healing process leading to hardening of the native tissue and "scarring" within the affected organ. In principle, this process could affect any organ system and very often leads to disruption of organ function<sup>3</sup>. Connective tissue cells play a key role in normal wound healing in healthy individuals, but in diseased tissues and surgically traumatised wounds the process of wound healing can be over activated

due to various factors such as genetic, immune related and infection. This results in activation of connective tissue cells that cannot be switched off, forming fibrotic tissue. This can lead to an enormous amount of matrix deposited in the tissue, leading to scarring and dysfunction of the affected tissue. In the nose and para-nasal sinuses it results in the loss of ciliary function or blocked pathways of mucosal clearance. In the abdomen it is obstruction of the bowel caused by the fibrotic bands, while in the spine fibrotic tissue causes compressive neurological dysfunction leading to persistent pain and dysfunction after surgery.

Chitin is a derivative of glucose, fibrous in nature consisting of polysaccharides and is a major constituent in the exoskeleton of arthropods and the cell walls of fungi, including hard outer skeleton of shellfish, crab, lobster, and shrimp. It is biocompatible and biodegradable<sup>4</sup> and has been widely used by food and beverage industry, fashion and now in the medical field. In our studies we use the hydrogel form of Chitosan. In this form it is succinylated and combined with dextran aldehyde to form a post-operative wound dressing that reduces scar formation but can also act as a drug carrier for treating drug resistant organisms.



**Figure 1: Schematic representation of the possibilities of processing chitosan into different forms for medical use**

## Wound Healing

### 1.1.1 Historical Perspective

Caring for the wound is an ancient art practiced by human beings and has been found in Mesopotamian tablets 2200BC and later the Egyptian papyrus in 1400BC. In these ancient texts honey, grease, and lint was used to dress open wounds to remove dead tissue and pus to encourage wound healing <sup>5</sup>. The Hippocratic Collection discusses the ancient (400 BC) Greek practice of surgical drainage of pus and in the middle ages the Bible describes “the Samaritan soothed his wounds with olive oil and wine and bandages”. Oil may have provided some protection from infection as bacteria grow poorly in oil, and oil would have prevented the bandage from sticking to the wound as a non-adherent dressing. Wine (which has an alcohol content of around 10%) is more bactericidal than 10% alcohol. Wine's antibacterial components are the pigments malvoside and oenoside. Ancient salves used a mixture of one-third honey and two-thirds grease (butter) and have been shown to decrease a  $10^5$  count of *Staphylococcus* and *Escherichia coli* to less than  $10^2$  within 24 hours<sup>5</sup>.

Haemostasis is a necessary step for wound healing, was vaguely practiced by Hippocrates but came into modern usage in 1674 when reintroduced by French Army surgeon Etienne J. Morel with the use of the tourniquet<sup>6</sup>. Ambroise Paré (1510-1590) a clinician in the 16th century is often quoted, “I dressed the wound; God healed it”. He cared for battlefield wounds by using "oyle of Elders scalding hot" and cauterizing the wounds. He performed amputations as treatment for gunshot wounds<sup>7</sup>. This was an accepted form of wound care until 1865, when Dr. Joseph Lister (1827-1912) first demonstrated the benefit of anti-sepsis in surgery by his treatment of wounds with dressings soaked in carbolic acid. This was the beginning of the germ theory and infection being understood. Modern practice of wound care with debridement is credited to Belgian Army surgeon Antoine Depage (1862-1925)<sup>5</sup>. It was

Depage who often found that the wound environment was fertile ground for the growth of pathogens and causing gangrene. At this time Alexander Fleming was doing many of his bacteriologic studies in the research laboratories of Depage's military hospital at La Panne and identified *Bacillus aerogenes capsulatus* (*Clostridium perfringens*, *Clostridium welchii*), *Clostridium tetani*, streptococci, and staphylococci<sup>5,8</sup>.

Ignaz Semmelweis (1818-1865), an unrecognised Hungarian surgeon discovered sepsis as cause for wound infection and death. This was later espoused by the great scientist Louis Pasteur (1822-1895) but the surgery world only accepted this after Joseph Lister (1827-1912), working in Scotland, discovered the value of antisepsis<sup>7</sup>. Several others in the 20<sup>th</sup> and 21<sup>st</sup> century have contributed to the biology of wound healing. Jerome Gross a scientist/physician discovered collagenase while studying tadpole development during metamorphosis. Together with Charles Lapierre, a Belgian dermatologist, working in his laboratory, and Harvard surgeon Hermes Grillo, they studied the process of wound contraction in great detail. Grillo then took this knowledge to the operating room, where he devised procedures to correct and prevent recurrent tracheal stenosis. In the late 1950s, a young academic plastic surgeon at the University of North Carolina, stimulated by the work of Gross and others, began a quest to discover how to control scarring and transform the biology of wound healing into what he termed “surgical biology.” At the same time Stanley Cohen discovered the first epidermal growth factor (EGF) growth factor, EGF and Anita Roberts discovered transforming growth factor (TGF- $\beta$ ). The understanding of wound healing had entered the current era based on the historical concepts of managing trauma, haemostasis and antisepsis.

### **1.1.2 Surgical trauma and wound healing:**

Over the past 300 years historical texts have shown that the wound has an innate ability to heal (in the absence of infection and repeated trauma) and is able to control this process, largely through the local inflammatory cells. James Carrick Moore's "vis medicatrix naturae" dictated the philosophy of wound care in the early 19<sup>th</sup> century. Which stated "When any accident or disease injures the human frame, it was early observed, that the body possessed within itself, a power of alleviating or remedying the evil. In consequence of this power it happens, that whenever the structure or functions of any part of the body are disturbed, such operations are immediately excited as have a tendency to restore the machine to its former state"<sup>9</sup>, which essentially meant leaving it to nature or body's natural healing mechanism to take over and with minimal intervention from surgeons.

Surgical induced trauma in any part of the body can occur from any number of mechanical or thermal forces that lead to disruption of the skin and damage to the connective tissue and vasculature. Injury can be caused by blunt trauma due to instrument or tissue handling, a clean cut with knife or powered instruments such as shavers and drills or by thermal injury caused by burns or cauterization<sup>10</sup>. A wound is defined as an injury to the body that typically involves laceration or breaking of a membrane and damage to the underlying tissues<sup>11</sup>.

### **1.1.3 Wound Healing: Patho-physiology**

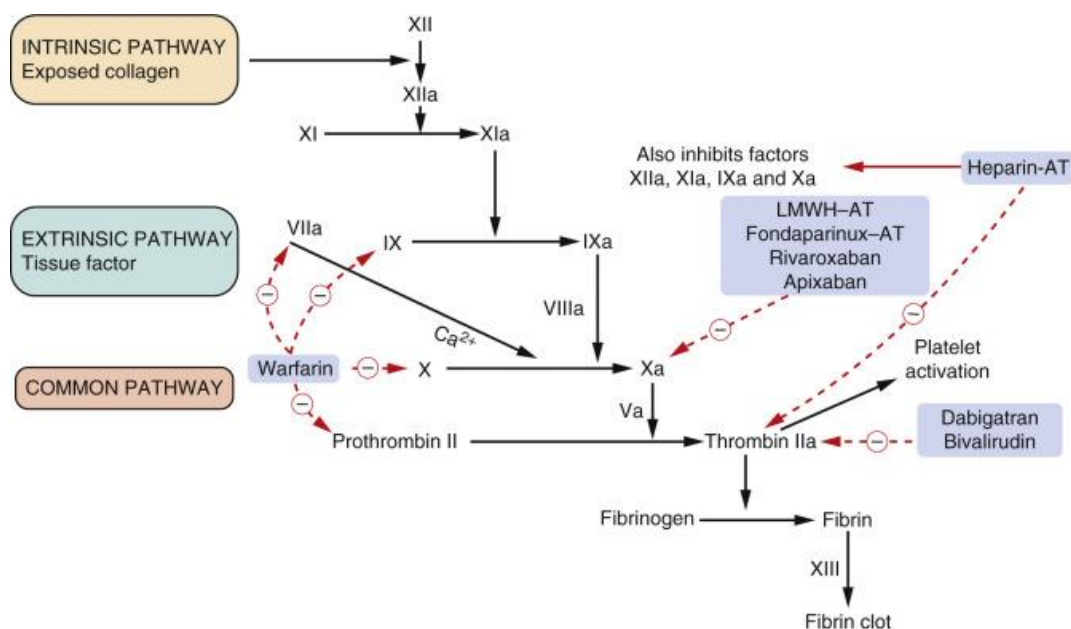
Trauma or a wound result in bleeding along with exposure of collagen, endothelium, and intravascular and extravascular proteins. This environment serves as a stimulus for haemostasis, the body's first step in the wound healing process, clot formation is enabled by chemokines and fibroblastic activity.



### 1.1.4 First Phase:Haemostasis and Inflammation:

Wound healing in general begins with haemostasis- a complex process involving vasoconstriction, plateletaggregation, bloodcoagulation. Vasoconstrictionis a reflexive saving reaction designed to limit the blood flow by release of endothelin, vasoconstricting circulating catecholamines (epinephrine) and prostaglandins from injured cells which in turn causes stimulus of the sympathetic nervous system (norepinephrine) with further vasoconstriction.

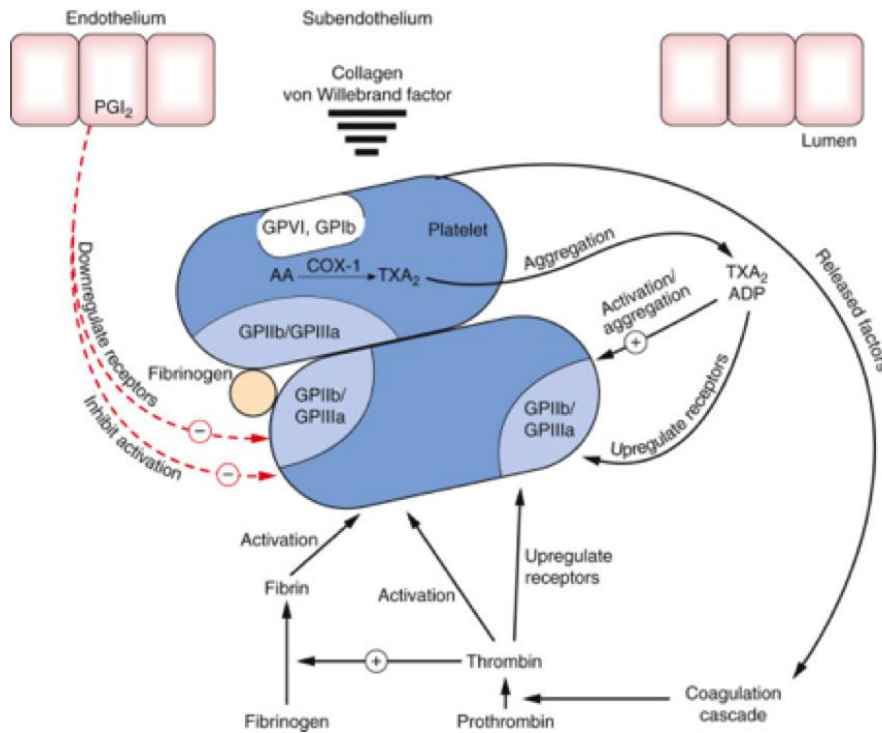
The clotting mechanism is driven by chemical triggers (von Willebrand factor (vWF) and collagen<sup>12</sup> that are released from the injured endothelium which otherwise when intact secrete prostacyclin and nitric oxide that inhibit platelet activation<sup>13</sup>. Platelets are the first responders in wound healing, when activated platelets contribute to haemostasis through the process of adherence, aggregation, and degranulation.



**Figure 2: Steps of Haemostasis; Intrinsic and Extrinsic Pathways Adapted from (13) with permission.**

Platelets are the first cells to respond in wound healing, activated platelets contribute to haemostasis through the process of adherence, aggregation, and degranulation. Exposed

collagen and thrombin at the site of injury comes in contact with blood flow, leading to stimulation of the circulating platelets causing adhesion. Platelet adherence is a result of interactions between platelet glycoproteins VI and collagen, glycoprotein Ib-V-IX complex and collagen-bound von Willebrand's factor.



**Figure 3: Activation of Platelets in clot formation, adapted with permission from (13)**

Tissue factor activates the extrinsic coagulation pathway leading to the production of thrombin an independent initiator of platelet activation. Thrombin interacts with a receptor on the platelet surface leading to the release of Adenosine diphosphate <sup>14</sup>, serotonin, and thromboxane A<sub>2</sub> causing platelet aggregation. This in the presence of fibrin matrix forms a clot or thrombus which in turn blocks the endothelial defect and stops the bleeding. This forms a layer which conserves degranulated substances from platelets. This is made up of numerous cytokines, growth factors, and matrix proteins stored within platelet alpha granules (Table 1). These substances promote a variety of cellular and extracellular mechanisms which promote the wound healing process including matrix deposition, chemotaxis, cell proliferation, angiogenesis, and remodelling.

**Table1: Platelet alpha granule components and their role in wound healing (14)**

| Adhesion Glycoproteins                                                                                                          | Proteoglycans                                                                                                                                                                                        | Haemostasis Factors & Cofactors                                                                                                                                    | Cellular Mitogens                                                                                                                                                                                                                                                                                                                                                                                                                     | Protease Inhibitors                                                                                                                                                                                                                                                                                                                                                  | Miscellaneous                                                                                                    |
|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"> <li>• Fibronectin</li> <li>• Vitronectin</li> <li>• Thrombospondin</li> <li>• vWF</li> </ul> | <ul style="list-style-type: none"> <li>• Platelet factor 4 (PF4)</li> <li>• Beta-thromboglobulin (<math>\beta</math>TG)</li> <li>• Serglycin</li> <li>• Histidinerich glycoprotein (HRGP)</li> </ul> | <ul style="list-style-type: none"> <li>• Fibrinogen</li> <li>• Factor V, VII, XI, XII</li> <li>• Kininogens</li> <li>• Protein S</li> <li>• Plasminogen</li> </ul> | <ul style="list-style-type: none"> <li>• Platelet derived growth factor (PDGF)</li> <li>• Transforming growth factor (<math>TGF-\beta</math>)</li> <li>• Endothelial cellgrowth factor (ECGF)</li> <li>• Epidemic growth factor (EGF)</li> <li>• Vascular endothelial growth factor (VEGF)</li> <li>• Vascular platelet factor (VPF)</li> <li>• Insulin like growth factor (IGF)</li> <li>• Interleukin-<math>\beta</math></li> </ul> | <ul style="list-style-type: none"> <li>• <math>\alpha_2</math> Macroglobulin</li> <li>• <math>\alpha_2</math> - Antitrypsin</li> <li>• Platelet derived collagenase inhibitor (PDCI)</li> <li>• <math>\alpha_2</math> - Antiplasmin</li> <li>• Tissue factor pathway inhibitor (TFPI)</li> <li>• Platelet inhibitor factor (PIXI)</li> <li>• C1 inhibitor</li> </ul> | <ul style="list-style-type: none"> <li>• IgG, IgA, IgM</li> <li>• Albumin</li> <li>• Glial/multimerin</li> </ul> |

Fibrin is a fibrous protein and forms a key component of the coagulation cascade. It is sourced from soluble circulating fibrinogen. Fibrin mesh is critical for establishing haemostasis following injury along with platelets. Fibrin has multiple binding sites for cells and growth factors that promote platelet spreading, cell infiltration, fibroblast proliferation, and angiogenesis. Fibrin degradation products also play a functional role in the wound repair process by encouraging cell infiltration and subsequent tissue remodelling of wound. Enhancing clot formation with substances derived from harvested fibrin or those that mimic fibrin can improve wound healing and also regulate the amount of tissue factor in the matrix.

**Inflammation:** Clot or thrombus formation initiates the next phase of wound healing, i.e. Inflammation. This stage is mainly driven by chemokines (or chemotactic cytokines). These are small heparin binding proteins that recruit circulating inflammatory cells to the injury sites via interaction with specific membrane-bound receptors.

In the first 48 hrs *Neutrophils* are the predominant cell type in the inflammation phase after which monocytes take over the wound healing as they mature into tissue macrophages. Chemokines IL-8 released by neutrophils attracts the macrophages and other cells to the wound site. CXC chemokines primarily attract neutrophils and lymphocytes(CXCL7 [neutrophil-activating peptide-2 (NAP-2)])<sup>15</sup>, and are believed to orchestrate the early phases of wound healing.

*Monocytes* migrate to the wound site and become macrophages who then play a central role in both the inflammatory phase and all stages of repair. Macrophages act as scavengers by phagocytosing debris and bacteria, and orchestrate inflammatory cytokines (including growth factors) such as TNF, IL-6, IL-1, bFGF, etc. IL-1 stimulates the proliferation of inflammatory cells and promotes angiogenesis through endothelial cell replication. TNF- $\alpha$  is a mitogen for fibroblasts. bFGF is a chemotactic and mitogenic factor for fibroblasts, endothelial cells and other mesenchymal cells, and in turn provides the stimulus for angiogenesis. In addition, bFGF stimulates wound contraction, epithelialization and production of collagen, fibronectin, and proteoglycans. Macrophages also secrete collagenases and elastases, which break down injured tissue and debride the wound<sup>16</sup>.

### **1.1.5 Second Phase: Proliferation**

Post injury day 4-12, proliferation or constructive phase begins, and 3 major activities take place during this period, namely matrix deposition, angiogenesis, and epithelialization.

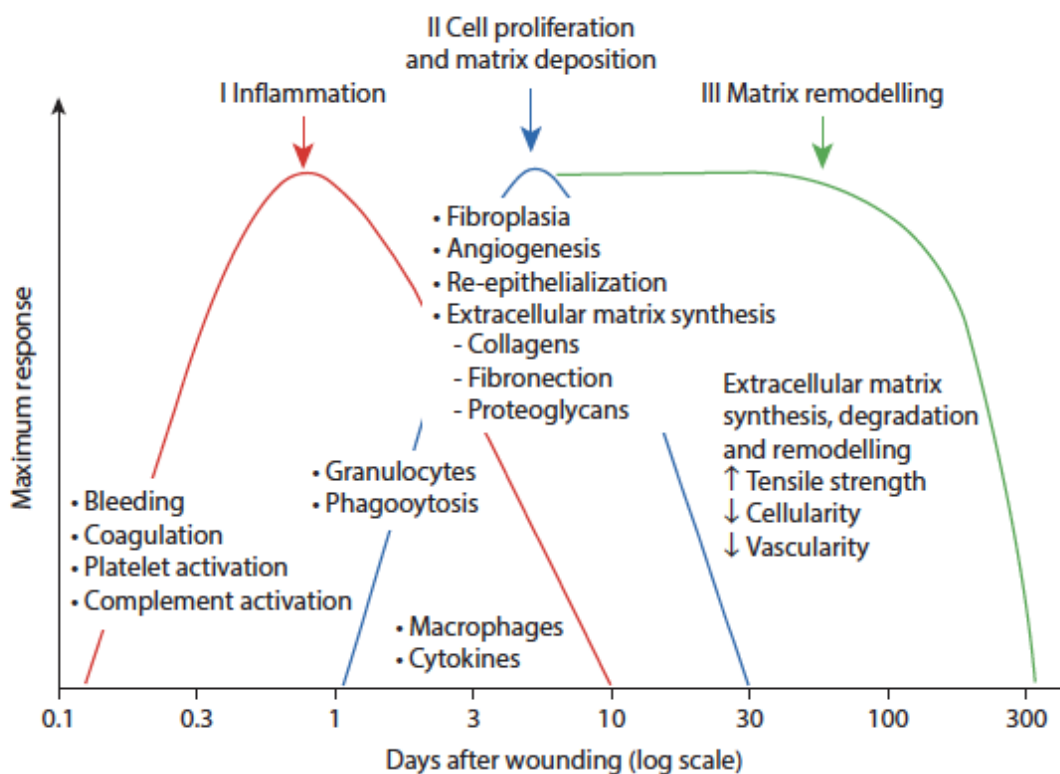
In this period, fibroblasts, smooth muscle cells, and endothelial cells infiltrate the wound and bridge the surgically created void over which epithelial cells begin to cover the site of injury<sup>10</sup>. This Phase is also characterised by the corresponding increase in blood flow into the damaged tissue by angiogenesis, which is new blood channel formation, seen as granulation tissue (a mix of fibroblasts, macrophages and neovascualture). Once the fibroblasts migrate into the area of wound, they switch their role to protein synthesis and this activity reaches its peak 21 days from injury.

During the same process, epithelial cells at the edges of the injury also begin to proliferate and try to reach the counterpart on the opposite side attempting to close the wound. In the case of abdomen, the entire breach in continuity of endothelium is filled by proliferation of cells in the form of membranous sheet. Each tissue on the body has a set rate at which they regenerate. This can be controlled by various internal and external factors.

### **1.1.6 Third Phase: Remodelling**

The last stage of wound repair begins 14–21 days after injury and this can go for a year or more. In this period all the active injury related activities have ceased, and the extra cellular matrix<sup>17</sup> is gradually replaced by type I collagen, the predominant constituent of the normal human dermis. The healed tissue shrinks to a smaller size due to reduced blood supply and contraction of myofibroblasts<sup>18</sup>, and is composed of 80% to 90% type I collagen and 10% to 20% type III collagen. This is in contrast to the early phase of healing, wound matrix is weaker due the presence of 30% type III collagen<sup>10</sup>. In addition there are other host of external and internal factors that affect the wound healing<sup>19</sup> including the production of elastin fibres with proteoglycans within in the ECM. This will eventually affect the tensile strength of the scar.

In some fibroproliferative disorders, this process can be prolonged and can clinically recognized as hypertrophic scar<sup>20</sup> formation, adhesions or fibrous bands. This is due to proliferation of fibroblasts, with excessive deposition of fibroblast-derived ECM proteins and collagen<sup>19</sup>. Inflammation is key to normal wound healing, in adhesions or hypertrophic scars excessive inflammation results in abnormal healing<sup>21</sup>. This could be a result of hematoma or infection leading to recruitment of neutrophils and macrophages, producing inflammatory cytokines and reactive oxygen species (ROS) followed by fibroblast migration and proliferation into the wound are critical factors in these processes<sup>21,22</sup>. HTS and adhesive bands negatively impact the outcome of surgery in abdominal surgery<sup>23-25</sup>, spinal surgery<sup>26,27</sup>, and ENT surgery<sup>28-30</sup>.



**Figure 4: Figure 4 Adapted from Scott-Brown et.al representing the various phases of wound healing and cellular recruitment (29)**

### **1.1.7 Types of Wound Healing**

There are mainly 3 types of wound healing

1. Primary healing (healing by first intention in wounds with approximated wound edges)
2. Secondary healing (healing by secondary intention where the cut edges are not approximated)
3. Tertiary healing (wound edges are deliberately left unopposed due to infection or other reasons)

Healing by first intention is when cut edges are closely approximated to each other and wound heals within 12–24 hours. These wounds are clean and well perfused as in a surgical excision or a clean laceration. Wound edges are approximated using sutures, skin glue, steri-strips or other mechanical devices/instruments. Healing by secondary intention is when the cut edges are wide apart either due to loss of tissue or infection and wound edges come together by myofibroblasts. Tertiary wound healing occurs in wounds which are deliberately left open without approximating the wound edges due to infection and or soiling in abdominal wounds and usually approximated after 3-4 days.

### **1.1.8 Important factors that affect Wound Healing**

1. Age
2. Genetics
3. Surgical technique
4. Infection
5. Hypoxia

6. Nutrition
7. Immunosuppression
8. Chronic Disease

All these factors affect the outcome of the wound healing in different ways<sup>19</sup> and impairment in any of these determines the final outcome of the wound either individually or in combination. Different surgical situations have different combinations of the above factors, causing poor wound healing.

### **1.1.9 Complications of wound Healing**

Excessive scarring or adhesions after surgery are common. This is often from excessive bleeding resulting in clot formation and a framework allowing a fibrinous bridge to occur. Studies suggests infections either acute or chronic increase the likelihood of these fibrinous band forming post operatively. Whilst the outcome in sinus surgery would be a narrowing of the air passage, the same fibrous band could cause compression of sensitive neural tissue in the spine after spine surgery or form bands that obstruct the bowels in the abdomen causing dysfunction, infertility and even death if untreated.

There has been research through the ages to find agents that improve healing and scar formation. Historically, these have been designed to reduce bleeding, infection and loss of function with the help of various agents and methods (as listed in table 2).



### 1.1.10 Adjuncts in Wound Healing

**Table2: adapted from (30) Historical aspects of topical wound agents**

| Agents Used                                                                          | Surgeon                     |
|--------------------------------------------------------------------------------------|-----------------------------|
| Olive oil, honeycomb, gum Arabic, incense                                            | Johannes DeKetham, 1491     |
| Zinc ointments, alum, sal ammoniac, turpentine                                       | John Bell, 1810             |
| Oil or wax, honey, copper sulphate, mercurial salts                                  | Dominique Jean Larrey, 1814 |
| Wine, lead acetate                                                                   | Astley Cooper, 1825         |
| Zinc sulphate, lead acetate, copper acetate, mercuric chloride                       | James Syme, 1832            |
| Mercuric chloride, ammonium chloride, mercury and zinc cyanide, antiseptic treatment | Joseph Lister, 1884–1889    |
| Sodium hypochlorite, epicutaneous treatment                                          | Alexis Carrell, 1910        |
| Silver foil, mercuric chloride, sodium hypochlorite                                  | William Halsted, 1883–1917  |
| Sulphonamide, penicillin, sodium hypochlorite, allantoin                             | Hamilton Bailey, 1947       |
| Sulphonamide, penicillin, acetic acid                                                | George Crile, 1947          |

Research has a number of methods that have been used to reduce surgical scars such as pressure therapy<sup>31</sup>, intralesional corticosteroids<sup>32</sup>, bleomycin<sup>33</sup>, laser therapy<sup>34</sup>, intralesional interferon<sup>35</sup>, silicone gel sheeting<sup>36</sup> and onion extract gel<sup>37</sup>. Of these the most commonly used are pressure on the wound, silicone support and intralesional steroid. Pressure therapy is hypothesized to prevent scar formation by initially promoting haemostasis and then in the later phases of healing suppressing collagen production by restricting nutrient supply. Silicone have limited beneficial effect on skin scars because of their potential to cause a rash, pruritus and excessive sweating<sup>38</sup>. Intralesional injections are second-line therapies for the treatment of hypertrophic scars in the skin, Corticosteroids and bleomycin are hypothesized to inhibit the inflammatory process and expression of genes related to collagen and

glycosaminoglycan synthesis, decreasing fibroblast proliferation. Intralesional steroid injections are highly responsive (50% to 100%), indicating a profound effect of reducing inflammation on limiting hypertrophic scar formation<sup>39,40</sup>. However, 63% of the patients experience side effects, especially in the form of hypopigmentation, skin and subcutaneous fat atrophy and some experience telangiectasia<sup>41</sup>.

Chitosan has recently been used in multiple wounds in the body as it is a bio-compatible wound healing adjunct that is able to conform itself to shapes and forms that is required for the area of injury/wound and is able to be cleared by absorption within the body.

## **Chitogel**

### **1.1.12 General Properties:**

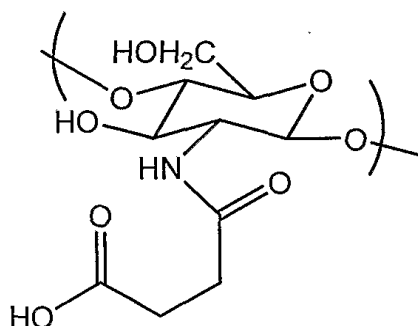
Chitogel is a polysaccharide hydrogel formed between succinyl-chitosan and dextran aldehyde (dissolved in a sodium phosphate buffer). Succinyl-chitosan is a polymer of chitosan which is produced by the hydrolysis of chitin, found in shellfish. Both chitosan and chitin as a group make up the second most abundant polysaccharide occurring in nature (after cellulose). Chitosan has been widely applied in a variety of settings including use in:

- (1) Foods as a preservative and antimicrobial agent
- (2) Agriculture as an adhesive agent on seeds, pesticides and fungicides
- (3) Cosmetics as a hydrating agent
- (4) Shampoos and toothpastes as a preservative agent

- (5) Medications as a weight and cholesterol lowering agent
- (6) The United States (US) Military as a haemostatic dressing
- (7) In wastewater management as a flocculent that absorbs greases, oils, metals, and other toxic substances

More recently chitosan has become recognised as having a potential role in biomedical and other more formal pharmaceutical applications. Its properties as an effective haemostat, anti-adhesive and pro-wound healing agent, as well as its antimicrobial action against a number of bacterial species has made it an attractive candidate for many medical and surgical applications and has been recently approved by FDA as a post-surgical device in sinus surgery. Non-clinical and clinical studies investigating both chitosan in general as well as Chitogel specifically are outlined in the sections to follow.

Succinyl-chitosan is supplied via the Department of Chemistry, The University of Otago (Dunedin, New Zealand). The raw material (chitosan) is sourced from Sigma-Aldrich Chemicals (Sigma-Aldrich New Zealand Ltd., Auckland, New Zealand). The solution is packaged as 10 ml samples in glass vials by The Department of Chemistry, The University of Otago then sent to The Department of Otorhinolaryngology, The University of Adelaide (The Queen Elizabeth Hospital site, Woodville, South Australia, Australia). It is extensively chemically devitalized and can be regarded as synthetic. It is a buff coloured solution with no odour. It has a molecular formula of  $(C_8H_{16}NO_7)_n$  [Figure 5] and a molecular weight of 450,000 Da. It is produced at a concentration of 5% in water with 0.3% sodium hydrogen phosphate buffer and has a pH of 7.4 (physiological pH). Succinyl-chitosan has a number of synonyms which include: Chitosan, N-(3-carboxy-1-oxopropyl), chitosan succinate, Chitosan succinamide, Chitosan succinyl amide, Hydrases, and N-Succinyl chitosan.



**Figure 5: The chemical structure of N-succinyl-chitosan (12)**

Succinyl-chitosan is non-flammable. It is incompatible with strong oxidisers and acids. Dextran Aldehyde is a white coloured solid powder with a slight odour. Its molecular formula is  $(C_6H_8O_6)_n$  and its molecular weight is 80,000 Da. It is designed to be dissolved in the sodium phosphate buffer. It has a neutral pH, however when mixed with the buffer solution, the pH is 7.4 (physiological pH). Dextran aldehyde is flammable; however it is mixed immediately prior to application into the sino nasal sinuses with non-flammable sodium phosphate buffer to form a solution, then with non-flammable succinyl-chitosan to form a gel, therefore its flammable risk is minimised. It is incompatible with strong oxidisers and acids.

The sodium phosphate buffer solution is an isotonic fluid in which the dextran aldehyde is dissolved. It is produced at a concentration of 0.3% in water and has a pH of 7.4.

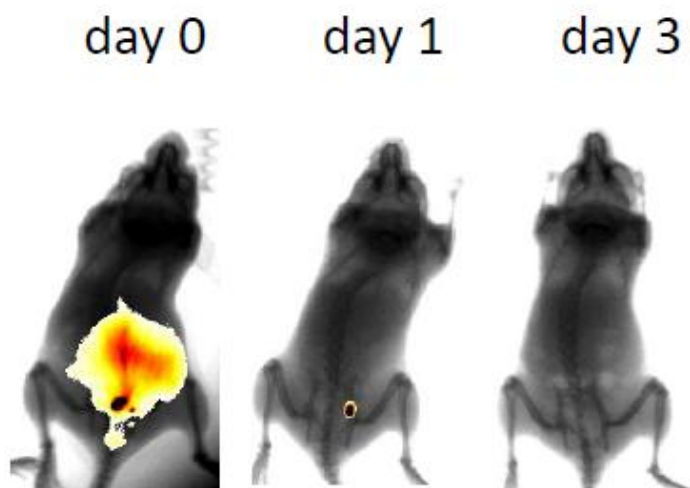
### **1.1.13 Pharmacokinetics and Toxicology**

Specific to Chitogel, The Department of Chemistry, The University of Otago (Dunedin, New Zealand) had conducted an animal toxicology study<sup>42</sup> where radiolabelled-Chitogel and control (normal saline) was injected intra-peritoneally into BALB/c mice to determine three factors:

- (1) the biodistribution/degradation rate of Chitogel
- (2) the clearance rate of Chitogel from the intact physiological rat system and
- (3) to assess the pro-inflammatory response of Chitogel.

Mice were monitored over a 6-day period where faeces samples were collected to measure radioactivity levels and Tritium was detected in faeces, organs, tissues, and serum of mice intraperitoneally injected with Chitogel. The study showed that majority of the Chitogel biodegraded within 3 days and biodistribution of its metabolites were highest in the spleen, followed by the organs of excretion: the liver and kidney. These results suggest that Chitogel is well-tolerated within the intraperitoneal space and is eliminated into non-toxic waste products in a timely manner (by Day 6).

As a follow-on study, The Department of Chemistry, The University of Otago (Dunedin, New Zealand) conducted a similar study using the same protocol in BALB/c mice, but with subcutaneous rather than intraperitoneal injections<sup>42</sup>. They also added another arm to the study: a positive control with lipopolysaccharide (LPS) to induce inflammation. The follow-on study assessed 5 time points: 2 hours, 4 hours, 1 day, 3 days and 7 days. Fluorescence imaging of Chitogel showed that the steady degradation over a 3-day period [Figure 6]. There was no fluorescence detected in mice imaged on day 6.

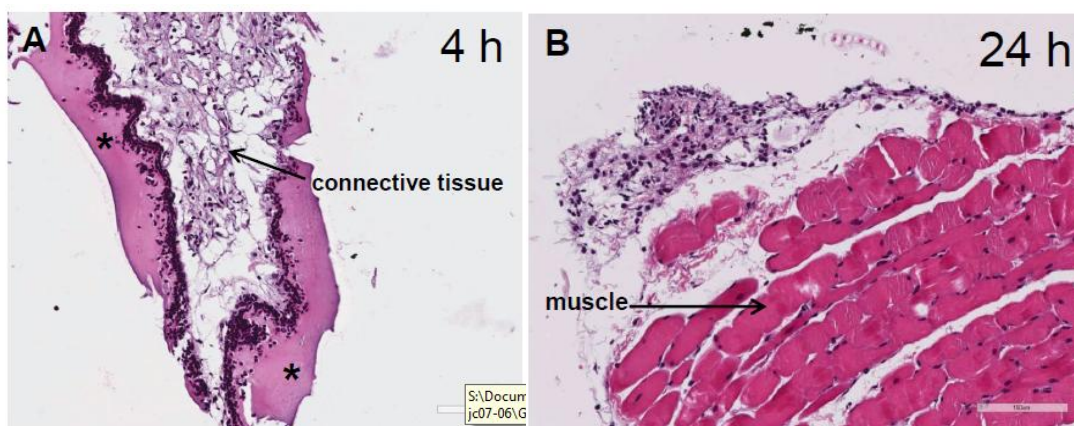


**Figure 6: XR and NIR-fluorescence imaging of mice injected with Chitogel(42)**

The general well-being of Chitogel-injected mice was excellent. No toxic response or death occurred during the 6-day observation period. In that study, Chitogel-injected mice showed no statistically significant differences in any of the following serum indices: total protein , albumin, alanine aminotransferase, creatinine phosphokinase, creatinine, urea and globulin as an indicator of inflammatory response.

To investigate the in-vivo inflammatory response resulting from Chitogel, the following pro-inflammatory cytokines: TNF- $\alpha$ , IL- 1b, IL-6, IL-12, anti-inflammatory cytokines, IL-10 and IL-13 and chemokines, macrophage inflammatory protein (MIP) 1a and 1bwere measured. LPS-injected mice had statistically significant increases in the amount of both pro-inflammatory cytokines as well as anti-inflammatory cytokines released as compared to saline and Chitogel-injected mice. The chemokines measured followed the same trend as the pro-inflammatory and anti-inflammatory cytokines. There was no statistically significant difference between saline and Chitogel-injected mice. These results indicated that subcutaneously - injected Chitogel did not affect the blood system, liver or renal function, nor was there significant inflammation in muscle tissue, heart, or brain. As well, Chitogel did not elicit an inflammatory response when compared to the LPS positive control.

This second study also investigated Chitogel distribution and degradation in vivo at the same time points. The concluding remark was that there was no toxic response or histological changes due to subcutaneous injection Chitogel in mice compared to control [Figure 7]. Cytokine and chemokine levels were consistent with histological examination of tissue around the injection site with >80% of the mice injected showing no inflammation. There were no signs of chronic inflammation in any mouse. Thus, the results from this second study suggest that the gel is well-tolerated within the subcutaneous space and exhibited acceptable biocompatibility for practical use in in vivo applications.



**Figure 7: Images of H&E stain of surrounding tissues at the Chitogel subcutaneous injection site at (A) 4 hours and (B) 24 hours post injection in mice. \*Indicates Chitogel(43)**

Again, the general well-being of the Chitogel-injected mice was excellent. No toxic response or death occurred during the 7-day observation period. The animals displayed normal energy, normal behaviour, free movement, and shining hair. There was no flare or ulceration in the skin, no mouth, nose dryness or oedema; as well as no vomit, running nose or eye secretion. Animal faeces were in regular form and normal colour, without mucus, pus, or blood.

To support further the safety of Chitogel, animal toxicology studies evaluating chitosan alone is summarized in the table below which indicates the animal species, chitosan product, concentration and main outcome/conclusion(s) [Tables 3 and 4]

**Table3: Animal toxicology studies evaluating chitosan**

| Report number | Title                                                                                        | Animal species      | Chitosan products              | Concentration                                                                                     | Conclusion                                                                                                                                                |
|---------------|----------------------------------------------------------------------------------------------|---------------------|--------------------------------|---------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| 658/543       | Single dose toxicity study by the intravenous route                                          | Rat                 | PROTASAN UP G 113 (2.8 mPas)   | 20, 50, 100 mg/kg in preliminary, 25 mg/kg in main study                                          | No mortality at 20 or 25 mg/kg, 2/2 and 1/2 deaths at 50 and 100 mg/kg, resp. Lower body weight on day 1-2, same as control after.                        |
| 658/557       | Single dose toxicity study by the intravenous route                                          | Rat                 | PROTASAN UP CL 113 (5 mPas)    | 25, 50, 100 mg/kg in preliminary, 50 mg/kg in main study                                          | No mortality at 25 and 50 mg/kg in preliminary study. Mortality was 1/10 treated animals in main study at 50 mg/kg.                                       |
| 658/541       | Sensitizing potential in the guinea pig. Magnusson & Kligman test (GPMT)                     | Guinea pig          | PROTASAN UP G 213 (91 mPas)    | 1 mg/kg intradermal, 60 mg/ml topical occlusive induction. 60 mg/ml topical challenge             | No mortality observed. Body weight not influenced. Induction: moderate irritation at injection site. After challenge no sign of delayed hypersensitivity. |
| 658/556       | Sensitizing potential in the guinea pig. Magnusson & Kligman test (GPMT)                     | Guinea pig          | PROTASAN UP CL 113, (5 mPas)   | 0.5 mg/ml intradermal, 60 mg/ml topical occlusive induction. 60 mg/ml topical occlusive challenge | No mortality. No influence on body weight. Signs of irritation were noted during induction. No delayed hypersensitivity in test animals.                  |
| 658/542       | Evaluation of the potential to induce immediate hypersensitivity: induced anaphylactic shock | Guinea pig          | PROTASAN UP G 213 (91 mPas)    | 10 mg/kg subcutaneous induction, 20 mg/kg intravenous challenge                                   | No mortality observed. Body weight similar in all groups. Cyanosis noted in 5 treated animals within 1 hr of challenge, cleared by 4 hr.                  |
| 10396         | Bacterial reverse mutation test                                                              | Plate incorporation | PROTASAN UP G 213 (75 mPas)    | ≤5000 mg                                                                                          | No significant increase in numbers of revertants. 5000 µg/plate toxic to TA98 and TA 1537 (± activation).                                                 |
| 507340        | Acute dermal irritation test                                                                 | Rabbits             | PROTASAN UP B 80/20 and 80/500 | 0.5 g                                                                                             | No edema or erythema. Non-irritant.                                                                                                                       |
| 507356        | Local lymph node assay (hypersensitivity)                                                    | Mouse               | PROTASAN UP B 80/20 and 80/500 | 1, 2.5, 5%<br>0.5, 1, 2%                                                                          | No adverse clinical signs. No Simulation Index over 3 = no sensitizing effect.                                                                            |



**Table4:Animal toxicologystudiesevaluatingchitosan (continued)**

| Report number | Title                                                                             | Animal species                                                  | Chitosan products                                           | Concentration                   | Conclusion                                                                                                                                                                   |
|---------------|-----------------------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ALG-95-001    | Effect of chitosan salts on cell survival of V-79 and 3T3 cells cultured in vitro | In vitro cell culture 3T3 mouse fibroblasts V79 Chinese hamster | PROTASAN UP G 213 (84 mPas)<br>PROTASAN UP CL 113 (12 mPas) | 0-1 mg/ml<br>24 hr exposure     | Little or no effect at up to 1 mg/ml. Reduction in cell survival by 15% (CL) and 35% (G) at 5 mg/ml.                                                                         |
| 658/525       | Single dose toxicity study by the intraperitoneal route                           | Mouse                                                           | PROTASAN UP G 213 (84 mPas)                                 | 100, 250, 500 mg/kg             | No mortality. No abnormal clinical signs, normal weight increase.                                                                                                            |
| 658/526       | Single dose toxicity study by the intraperitoneal route                           | Rat                                                             | PROTASAN UP G 213 (84 mPas)                                 | 100, 250, 500 mg/kg             | No mortality. No abnormal clinical signs, normal weight increase.                                                                                                            |
| 658/527       | Single dose toxicity study by the intraperitoneal route                           | Mouse                                                           | PROTASAN UP CL 113 (12 mPas)                                | 100, 250, 500 mg/kg             | No mortality. No abnormal clinical signs, normal weight increase.                                                                                                            |
| 658/528       | Single dose toxicity study by the intraperitoneal route                           | Rat                                                             | PROTASAN UP CL 113 (12 mPas)                                | 100, 250, 500 mg/kg             | No mortality. No abnormal clinical signs, normal weight increase.                                                                                                            |
| 658/539       | 13 week oral (gavage) toxicity study                                              | Rat                                                             | PROTASAN UP G 213 (91 mPas)                                 | 100, 300, 600 mg/kg             | No treatment related deaths. No differences in body weight nor food consumption. No abnormal clinical signs, normal weight increase.                                         |
| 658/540       | 7 day intranasal tolerance                                                        | Rat                                                             | PROTASAN UP G 213 (91 mPas)                                 | 0.5 and 1 mg admin. x 3 per day | No mortality during study. Treated animals have some increase in mucus production, no clear dose-effect seen. No abnormal clinical signs, normal weight increase.            |
| 658/538       | Single dose toxicity study by the intravenous route                               | Rat                                                             | PROTASAN UP G 213 (91 mPas)                                 | 25 mg/kg                        | No mortality was observed. Subdued behaviour up to 30 minutes after injection. Treated animals lost weight between days 1 and 2, thereafter weight gains similar to control. |

Chitogel has inherent haemostatic and anti-adhesive properties and an excellent safety profile. Both in vitro studies and in vivo animal and human randomised trials have shown that Chitogel achieves haemostasis significantly quicker than control <sup>43,44</sup>, and reduces the incidence of post-surgical adhesions in endoscopic sinus surgery <sup>45,46</sup>.

### 1.1.14 Chitosan as a haemostatic agent:

Chitosan has a long history of being used as a haemostatic agent:

- (1) Wedmore et al <sup>47</sup> described a chitosan patch that is distributed to over 400,000 military personnel with significant improvement in haemostasis when applied to a wound and no adverse effects attributed to the chitosan patch.

- (2) A chitosan patch has been marketed for use in dental extractions<sup>48</sup> with claims of significant improvement in haemostasis and no adverse or allergic events since its introduction in 2003.
- (3) Valentine et al (2009)<sup>49</sup> conducted a study in 21 sheep infected with *Oestrus* that underwent standardised mucosal injuries in the sinuses. They evaluated the bleeding scores at Time = 0 (at time of mucosal injury) and every 2 minutes in Chitogel-applied mucosa vs. control (nothing) mucosa. That study found that Chitogel was significantly more haemostatic at 2, 4 and 6 minutes after injury. The average time to haemostasis was significantly better in Chitogel sides compared to control sides (4.09 vs 6.57 minutes,  $p=0.049$ ). Furthermore, complete haemostasis occurred within 6 minutes for all Chitogel sides, whereas control sides were still bleeding at 8 minutes in 3 sheep and 10 minutes in 1 sheep.
- (4) Valentine et al (2010)<sup>50</sup> followed on from sheep to demonstrate the same outcome in 40 human subjects undergoing ESS for CRS in which patients were their own controls such that one side was randomised to receive Chitogel and the other served as control. Similarly, they scored baseline bleeding (Time = 0) immediately after the sinus operation, and every two minutes thereon. In that study, Chitogel achieved haemostasis within a mean time of 2 minutes (2-4 minutes) and compared with 10 minutes for the control sides. Wound healing evaluated in that study is discussed in the section below.
- (5) Valentine et al<sup>51</sup> then conducted another study in 20 sheep with standardised carotid artery injuries. Each sheep was randomised to 1 of 5 haemostatic techniques; of which 1 was Chitogel. The outcomes measures in that study was time to haemostasis, duration of time that MAP was  $<55$  mmHg, total blood loss, volume and survival

time. The findings of that study were that Chitogel was inferior to the muscle patch and U-clip anastomotic device, but superior to control. Those results suggested that Chitogel is more effective in low-flow mucosal bleeding than high volume, high pressure bleeding (as is the case with carotid artery injuries).

(6) Chitogel Hemocon patch<sup>48</sup>, Clo-Sur pad (Merit/Medtronic Scion)<sup>52</sup>, Chito-Seal<sup>53,54</sup>, Syvek Patch<sup>55</sup>, Rapid Deployed Haemostat (Marine Polymer Technology)<sup>56</sup>, Trauma DEX (Medford)<sup>57</sup> are all FDA-approved chitosan dressings for use as topical haemostatic dressings. Both the Hemocon patch and the Syvek patch have extensive scientific data with promising results in RCTs indicating effectiveness as topical haemostats. There have not been any adverse reactions due to these topical patches reported in any of these trials. In Japan chitosan is used as a dressing (Beschitin)<sup>58</sup> that is used specifically for post-operative wound healing following sinus surgery. This has some scientific data to support its use in the Japanese literature and to date no adverse events have been recorded with its use.

### **1.1.15 Chitosan as an anti-adhesive agent:**

Chitosan has a long history of being used as an anti-adhesive agent:

- (1) Diamond et al<sup>59</sup> showed chitosan gel used topically in a gynaecologic randomised controlled trial of 34 women did not result in any adverse events. Furthermore, there were lower grades, severity and extent of adhesions.
- (2) Kennedy et al<sup>60</sup> found that the topical application of chitosan in 20 rats resulted in a significant reduction of adhesion formation in abdominal and pelvic adhesions.
- (3) Costain et al<sup>61</sup> found that the topical application of chitosan in 56 rats resulted in a significant reduction of adhesion formation in abdominal and pelvic adhesions.
- (4) Vlahos et al<sup>62</sup> found that the topical application of chitosan in 44 rats resulted in a significant reduction of adhesion formation in abdominal adhesions.

(5) Zhang et al<sup>63</sup> found that the topical application of chitosan gel inhibited adhesion formation in 240 rats whilst chitosan film resulted in increased adhesion formation.

#### **1.1.16 Chitosan as a weight-loss and/or anti-cholesterol agent:**

Chitosan has been used in other medical applications:

- 1 Kaats et al<sup>64</sup> showed in a double blind randomised controlled trial of 150 women who were given placebo or 3 g of chitosan/day showed no adverse effects from chitosan. Some parameters of weight and lipid distribution were significantly improved in the chitosan group.
- 2 Wuolioki et al<sup>65</sup> showed in a randomised controlled trial of 51 overweight women who were given 800 mg chitosan or placebo for 8 weeks, that chitosan was well tolerated and no side effects were reported. As well, there was no change in serum levels of fat-soluble vitamins or serum iron/transferrin levels. However, there was also no significant reduction in weight between the two groups.
- 3 Bokura<sup>66</sup> showed in a randomised controlled trial of 90 female volunteers who received either placebo or 1.2 g chitosan daily for 56 days, that cholesterol levels were mildly reduced in the chitosan arm. Also, there were no adverse effects recorded.
- 4 Landes and Bough<sup>67</sup> showed progressive growth reductions in male Sprague-Dawley rats fed 10 or 15% chitosan diets for 8 weeks. They observed enlarged liver and kidneys with 15% chitosan diets.
- 5 Vahounyet al<sup>68</sup> showed no significant differences in weight gain in male rats fed 1 or 5% chitosan for 4 weeks. In rats fed with 5% chitosan, oleic acid and cholesterol absorption was lowered by 58% and 63% respectively.

- 6 Fukuda et al<sup>69</sup> found no significant differences in growth, food intake, liver weight, and dried faecal weight between control and 2% or 5% chitosan-fed male Wistar rats after 21 days on a low cholesterol diet. However, 5% chitosan diets did reduce serum cholesterol levels. Chitosan feeding also suppressed the formation of coprostanol in the intestines.
- 7 LeHoux & Grondin<sup>70</sup> showed that high-molecular-weight chitosan (>750 kDa) was a less effective cholesterol-lowering agent than a low molecular-weight chitosan (70 kDa). A 7.5% chitosan formula-maintained cholesterol homeostasis in male Long-Evans rats even with increased cholesterol intake.
- 8 Deuchi et al<sup>71</sup> showed that ascorbate had a synergistic effect with chitosan on the inhibition of fat digestion in male Sprague-Dawley rats.
- 9 Razdan et al<sup>72</sup> showed significant reductions of feed intake, body weight, total plasma cholesterol, and HDL-cholesterol in broiler chicks that were fed chitosan for 12 days.
- 10 Ormrod et al<sup>73</sup> showed inhibition of hypercholesterolemia and atherogenesis in gene knockout apolipoprotein E-deficient mice that were fed 5% chitosan for 20 weeks.

### **1.1.17 Chitosan as a drug delivery agent:**

The chemical structure of Chitosan facilitates drug delivery:

- (1) Illum<sup>74</sup> indicated that a chitosan gel influenza vaccine administered intranasally was successful in a human trial and was now introduced in Europe.
- (2) Chitosan has also been successfully trialled in mice, rat, guinea pig and bovine animal models as a drug delivery agent for various factors such as Growth Hormone, insulin, anti-angiogenesis peptide as well as influenza/diphtheria/pertussis vaccines.

Studies that have investigated chitosan for use orally (e.g. as weight and/or cholesterol-lowering agents) indicate that chitosan is well tolerated orally, with minimal side effects

(mild and transitory nausea and constipation in 5-6% of patients)<sup>66,75</sup>. A Cochrane review of 14 trials evaluating chitosan as a weight loss agent<sup>76</sup> suggests that: whilst there is some evidence for minimal weight loss with oral chitosan, it is safe for human consumption with no difference in the adverse events between chitosan and control groups.

### **1.1.18 Chitogel as a wound healing agent:**

Chitogel has been investigated in both animal and human models for its anti-adhesive properties. A prospective randomised controlled trial of Chitogel was performed in 20 sheep<sup>77</sup>. After creating standardised injuries and applying three types of haemostatic agents (Chitogel, SprayGel™ and recombinant tissue factor) compared to control in a randomised fashion, sheep were reviewed by a blinded observer monthly for four months. Results of that study showed that Chitogel was the only agent significantly superior to control in preventing adhesions. Chitogel was shown to reduce adhesions from 56% in control to 5% in the treatment group ( $p < 0.01$ ). Wound healing was determined by light and electron microscopy and measurements of ciliary beat frequency. The results of that study showed that Chitogel was significantly better (30% improved) than SprayGel™ group (13% improved), compared to control ( $p < 0.05$ ).

In the study mentioned above Valentine et al<sup>44</sup> investigated the haemostatic effect of Chitogel in 40 humans, adhesions and other factors of wound healing were also evaluated in the postoperative period up to 3 months post-ESS surgery. Following intra-operative bleeding evaluation, patients were followed up at 2, 6 weeks and 3 months where their adhesions, crusting, mucosal oedema, infection, and granulation tissue were graded. That study indicated that there was a significant reduction in adhesions post-ESS compared to control. There was no significant difference in the other parameters.

Ha et al<sup>46</sup> showed in 26 humans in a prospective, randomised, controlled trial of Chitogel vs. control (nothing), with each patient having intervention on one side with the other side serving as control, that Chitogel was significantly better in terms of preventing ostial stenosis post-ESS. The patients were evaluated at 2 weeks, 2 months and 3 months post-procedure by endoscopic measurements using a custom-designed ball probe. The results of that study showed that the frontal ostia maintained their diameters at 66% vs 31% for Chitogel vs controls. Similarly, the sphenoid ostia maintained their diameters at 85% vs 47%, and maxillary ostia at 74% vs 54% for Chitogel vs. control respectively (all  $p \leq 0.002$ ).

A follow-on study to these investigated the effect of Chitogel combined with Pulmicort Respules® (budesonide, 1 mg/2 mL) in the early post-operative period<sup>78</sup>. The budesonide solution was used for its anti-inflammatory properties in that study. Again, using patients as own controls, Chitogel and budesonide was compared with control (nothing) and steroid-only (betamethasone cream). Results have shown that Chitogel with budesonide is superior to both control and betamethasone cream at improving healing in the early post-operative period. In the frontal sinuses, sides treated with Chitogel and budesonide maintained 71% of their ostial diameter at 12 months, compared to 51% on the control side (no treatment). Similar results were seen in the sphenoid and maxillary sinuses, but less pronounced.

#### **1.1.19 Chitogel as an anti-bacterial/anti-biofilm agent:**

Bae et al<sup>79</sup> used chitosan as a mouth wash in 12 dental students for 6 weeks to show a significant reduction in bacterial biofilm growth and no adverse events. Paramasivam et al<sup>80</sup> investigated the anti-bacterial and anti-biofilm effects of Chitogel in an in-vitro study using fibroblasts isolated from human nasal tissue. They set out to determine the effects of Chitogel on (1) cell proliferation, (2) wound healing, (3) inflammation in fibroblast cultures

challenged with superantigen, *Staph aureus* enterotoxin B (SEB) and toxic shock syndrome toxin (TSST) (4). They found that Chitogel was highly effective at reducing IL-8 expression after TSST and SEB challenge, and also reduced non-challenged fibroblasts, indicating its anti-inflammatory effects on fibroblasts in the diseased state. Chitogel also showed strong anti-biofilm properties at 50% concentration and dextran on its own showed anti-biofilm properties at 1.25% concentration. Chitosan on its own reduced proliferation of fibroblasts to 82% of control and Chitogel reduced proliferation of fibroblasts to 0.04%. Furthermore, Chitogel significantly delayed epithelial cell defect closure rates over the first 2 days of wound-healing. In all trials that have been conducted involving Chitogel as a topical product in human subjects, there have been no adverse effects.

## Deferiprone (Def)

Def is an oral iron chelator used as a second line agent in thalassemia syndromes<sup>81</sup> when iron overload from blood transfusions occurs. There are multiple synonyms to Def (1,2-dimethyl-3-hydroxy-4-pyridinone, 1,2-dimethyl-3-hydroxypyrid-4-one, 1,2-dimethyl-3-hydroxypyridin-4-one, 3-hydroxy-1,2-dimethyl-4-pyridinone, CP20, Deferiprone, DMOHPO, Ferriprox<sup>82</sup>). Def as an iron chelator, has anti-microbial properties because of free radical scavenging and is known to improve wound healing (skin wounds)<sup>14</sup>. Scavenging ROS after abdominal surgery has been shown to significantly inhibit postoperative adhesion formation<sup>83</sup>. Iron being an enzymatic co-factor in biological systems becomes a target for bacterial cell wall synthesis. *Staph aureus* uses iron as oxidising and reducing agent in membrane bound cytochromes and has developed specific human specific haemoglobin receptors<sup>84</sup>. Chelation of the circulating Fe leads to depletion from the bacteria's surrounding environment, forcing the bacteria to up-regulate its iron transporters. The wound-healing activities of different concentrations of Def on primary human fibroblasts and primary human nasal epithelial cells



in air liquid interface culture (HNEC-ALI) were studied<sup>85</sup>. Along with this, effect of Def on fibroblast and epithelial cell migration, collagen production, ROS activity and potential for anti-inflammatory effects were also evaluated for its potential to limit hypertrophic scar tissue formation for future clinical applications<sup>85</sup>.

Pharmacokinetic studies<sup>86</sup> indicated rapid absorption in rats and monkeys. Elimination half-lives of deferiprone in rodents were similar to that observed in humans, while those in monkeys were slightly shorter. Serum elimination half-lives were independent of iron loading status. In vitro studies indicated glucuronidation by UGT1A6 as the major route of metabolism<sup>87</sup>. Repeat dose toxicity studies of 52 weeks duration were performed in both iron loaded and naive rats and monkeys. Maximum exposures were low; equivalent to that anticipated from a clinical dose of 75 mg/kg/day but estimated to be subclinical for a 100 mg/kg/day dose. No adverse findings were evident in the monkey study. In rats, the bone marrow, thyroid, adrenal and mammary glands were target organs for toxicity. Findings included bone marrow hypocellularity (with accompanying haematological changes), diffuse colloidal basophilia and hypertrophy of the thyroid follicular epithelium, hypertrophy of the adrenal zona fasciculata, and increased incidence and severity of mammary gland hyperplasia/fibroadenoma.

Regardless of iron status, Def at clinically relevant exposures, was genotoxic in a mouse micronucleus assay. No carcinogenicity studies were conducted with deferiprone. Aside from reduced oestrous cycling, no adverse effects on fertility were evident in either male or female rats at doses <0.5 times the clinical dose on a BSA basis. Based on long term rodent studies<sup>88</sup>, the proposed specification for the process impurity, maltol, in the drug substance was considered acceptable. The low tested doses and concentrations, which were in general estimated to be subclinical for the proposed higher dose of 100 mg/kg/day, limit the predictive value of negative findings and do not help to mitigate safety concerns.

## Gallium Protoporphyrin<sup>89</sup>

GaPP mimics haem in its molecular structure and is actively accumulated by bacteria via high affinity haem-uptake systems. The same uptake systems can be used to deliver antibiotic-porphyrin and antibacterial peptide-porphyrin conjugates<sup>90</sup>.

Ga(III) acts as an iron mimetic and is supposed to exploit Fe(III) transport systems to enter bacterial cells. Basic information on Fe(III) uptake and metabolism in bacteria are therefore instrumental to understanding the mechanism(s) of action and uptake routes of Gallium<sup>91,92</sup>.

The synonyms of GaPP are Ga-Protoporphyrin IX; Gape; Protoporphyrin I containing Ga; 7,12-Diethenyl-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoic acid, gallium complex. Its Molecular Formula: C<sub>34</sub>H<sub>32</sub>GaN<sub>4</sub>O<sub>4</sub> and Molecular Weight: 630.377 g/mol<sup>93</sup>.

Although gallium compounds show antimicrobial and antibiofilm activity against various bacteria, they have not found their way into clinical practice. There are two FDA approved gallium formulations on the market that are used as diagnostic agents in cancer therapy; these are the radioactive labelled gallium injections (i) gallium citrate Ga 67, FDA approved since 1976, and (ii) gallium dotatate Ga 68, FDA approved since 2016. The formulation Ganite (a gallium nitrate-citrate injection for cancer-related hypercalcaemia, FDA approved from 2003 to 2014) showed antimicrobial activity in vitro, however, this effect appeared species and strain dependent. Concentrations higher than the recommended dose would be required for a broad antibiofilm effect, raising toxicity concerns.

There is one gallium citrate formulation in the drug development pipeline, trade named Panaecin by Aridis Pharmaceuticals<sup>94</sup>. Clinical phase I studies commenced in November 2011 in the USA and indicated promising treatment efficacy in cystic fibrosis-associated respiratory tract infections after intravenous administration of gallium citrate. In January 2017, Aridis announced to progress into clinical phase IIa studies<sup>95</sup>.

To test for toxicity on mammalian cells, semiconfluent monolayers of four cell lines (MRC-5 primary human fibroblasts, Vero cells, MDCK cells and CaCO-2 cells) were incubated for 24 to 48 hours in the presence of 5, 25, 50, 250 and 400 µg/ml of Ga-PPIX dissolved in 0.02 M NaOH<sup>90</sup>. No detachment or rounding of cells was noticed in any of the monolayers. Preliminary toxicity testing on animals showed that a single intraperitoneal dose of 25 mg/kg of Ga-PPIX does not cause any hyperacute or acute effect on the health and behaviour of mice.

### **Combination of Def & GaPP:**

Def/GaPP combination has synergistic anti-microbial properties against MDR Gram-positive and Gram-negative bacteria. To further enhance Chitogel's anti-microbial properties, the team has developed a novel treatment combination (Def and GaPP) that has potent synergistic anti-microbial effects against Gram-positive and Gram-negative bacteria. Methicillin-Sensitive and Resistant *S. aureus* strains (frequent in surgical site infections) as well as MDR *E. Coli* and *P. aeruginosa* strains have been found to be highly susceptible to this novel treatment combination with eradication of planktonic and biofilm infections with extremely low dosages of Def and GaPP as low as 5µg/ml and 50µg/ml respectively. Def/GaPP combination is non-toxic in vitro. An LDH assay showed the absence of toxicity for both compounds alone and in combination for dosages up to 20mM Def and 500 µg/ml GaPP for 24 hours in L929 fibroblasts and bronchial epithelial (Nuli-1) cells (results not shown). Def has potent anti-adhesive properties - in addition to synergistically potentiating the anti-microbial effects of GaPP, Def has dose-dependent effects on fibroblast migration,

proliferation and collagen production in vitro (Fig. 8)AU2017900650, discovered here in the department of ENT, TQEH<sup>85</sup>.

Def/GaPP, when used in Chitogel has anti-microbial properties that are significantly more effective than commonly used antibiotics. Def and GaPP in Chitogel has a release profile over 2 weeks, this was measured using UV-V spectroscopy and HPLC (Figure 8). The gel provides a vehicle for the immediate and complete release of Def, with the maximal release occurring within 48-72 hours . GaPP had a delayed release profile with a maximal release of 16% after >72 hours. Combining Def and GaPP did not affect the release of either compound from the gel.

A comparison of anti-microbial effects of the Def-GaPP-Chitogel to the potent antibiotic Ciprofloxacin (Cip) and Chitogel control against different microbial biofilms (MSSA, MRSA, MDR *P. aeruginosa* and *E. Coli* clinical isolates and reference strains) was done. Whilst the sensitivity to different dosages and combinations of the compounds differed, all microbes tested showed a significant reduction in viability when treated with Def-GaPP-Chitogel compared to Cip-Chitogel and control Chitogel

In vitro data of Def-GaPP being nontoxic was studied by Richter, K. et al. wherein, cytotoxicity studies were performed on cell cultures by measuring LDH <sup>96</sup>. Def (20 mM) as a single treatment was tested on L929 and Nuli-1 cell lines. No statistically significant difference was observed between treatments and controls. Similarly, single treatment with GaPP had no significant effect on either cell lines at concentrations ranging from 100 to 400 µg/mall Only 500 µg/mL GaPP induced cell toxicity on both cell lines. Consecutive, dual treatment L929 cells were not sensitive to any of the tested concentrations in consecutive

treatments with 2 hours Def and 2 hours GaPP. In the Nuli-1 cell line, treatment with Def and GaPP reduced viability only at 500  $\mu\text{g/mL}$  GaPP<sup>96</sup>.

### Drug release:

Chitogel acts a good carrier for Def-GaPP with studies indicating that all Def was released from the gel within 48 to 72 h, while the release of GaPP gradually increased over time, reaching approximately 20% to 25% after 460 h within the medium. These release profiles were independent of drug concentrations in the gels (Def, 20 mM; GaPP, 100 [GaPP 100] and 500 g/ml [GaPP 500]). Interestingly, there was no statistical difference between the release of individual compounds and the release of the corresponding compounds from the combination gel.

**Determination of drug release kinetics:** 5 ml of Def-GaPP gel was prepared in a falcon tube(Fig 8a) and 10 ml of release medium (phosphate-buffered saline) was added(Fig 8b). This was incubated at 37°C on a rotating platform (70 rpm) for 20 days. Aliquots of 0.5 ml were taken at specific time points (0.5, 1, 2, 8, 16, 24, 48, 72, 96, 120, 170, 220, 290, 460 h) (Fig 8c) and replaced with fresh release medium. The concentrations of Def and GaPP were quantified by UV-visible spectroscopy at 280 nm and 405 nm, respectively, by interpolating from a standard curve.



Figure 8 a : 5 ml of Chitogel (drug-free gel, Def gel, GaPP gel, Def-GaPP gel, different concentrations)



Figure 8 b:10 ml PBS added

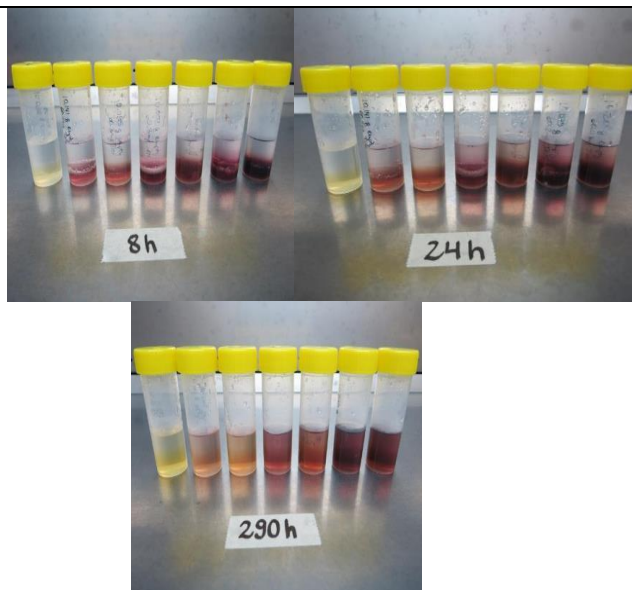


Figure 8 c: Release over time

### Figure 8: Determination of drug release kinetics

### Safety / Adverse effects and efficacy studies

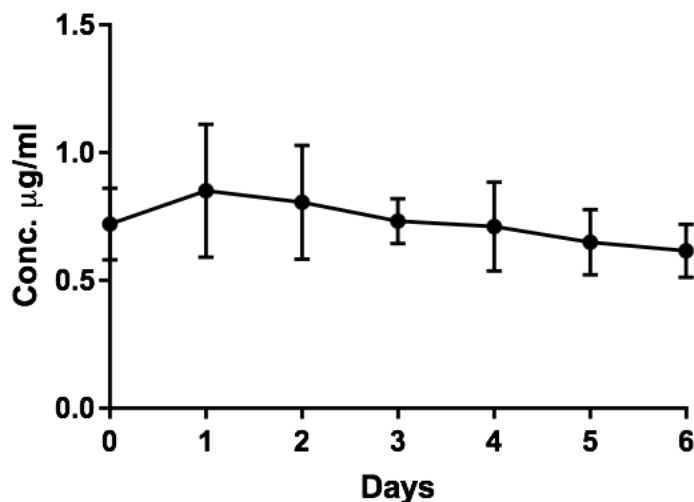
Def-GaPP-Chitogel is safe and effective in vivo. Preclinical studies were carried out to test the in vivo safety and efficacy of the Def-GaPP-Chitogel in a large animal-sheep<sup>43,77</sup> and infected wound model of porcine abdomen and sheep sinusitis. Results showed

- (1) absence of any toxic effects,
- (2) highly significant reduction of Staph aureus biofilms,
- (3) reduction in inflammatory cell counts and

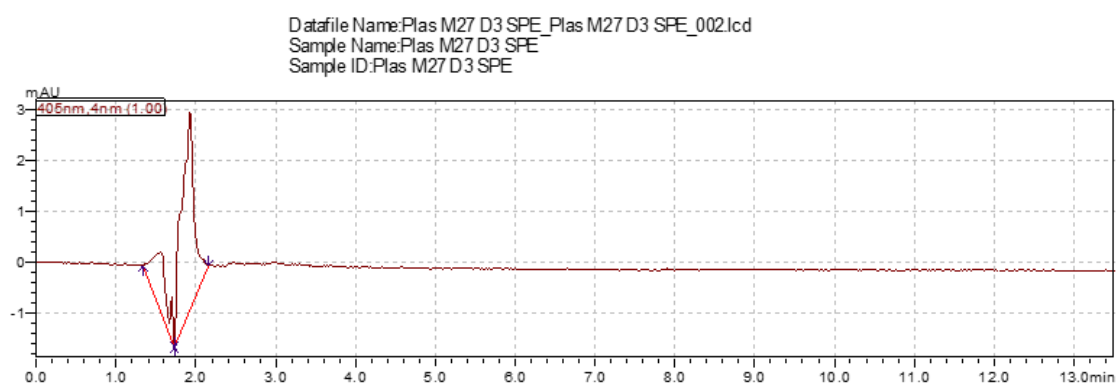
(4) improved wound healing in the Def-GaPP-Chitogel treated sheep compared to Chitogel treated control sheep<sup>97</sup>.

Using an established spinal surgery sheep model (non-infective model), pilot laminectomy study on 6 sheep to determine the anti-adhesion properties of the Def-Chitogel compared to Chitogel and untreated control were performed. Post-operative recovery and clinical examinations of the sheep were uneventful for all sheep over a 3-month period following surgery. MRI and histopathology showed absent toxicity and significantly reduced adhesion scores of paraspinal muscle fibres to the dura at three months post operatively (mean adhesion scores of 93.33% +/- 10.33 for the no-treatment control surgical sites compared to 86.67% +/- 10.33 for Chitogel compared to 66.67% +/- 10.33 for the Def-Chitogel treated sites,  $P=0.0076$ , Kruskal-Wallis). No effects were observed in bone or dura healing in any of the sheep, indicating Def-Chitogel reduced adhesion formation without negatively affecting the healing process of the dura. Together, these results strongly support the hypothesis that Chitogel loaded with Deferiprone and Gallium-Protoporphyrin is safe and effective to reduce adhesions after endonasal surgery.

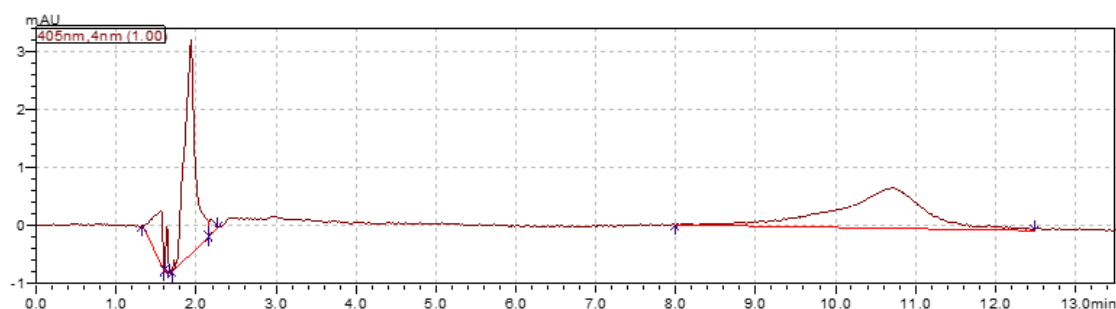
Serum level quantification in animal sinusitis model showed maximum Def concentration after one day (Fig 9) and no evidence of GaPP in the serum (Fig 10 A) in comparison to a GaPP spiked plasma (10 B).



**Figure 9:** Plasma concentration ( $\mu\text{g/ml}$ )  $\pm$  standard deviation of deferiprone (Def) over 6 days,  $n=4$ . The maximum Def concentration was reached after 1 day (0.85  $\mu\text{g/ml}$  Def) in the 4 sheep treated with Def-GaPP



**Figure 10A:** Example of a HPLC chromatogram of plasma samples. No gallium-protoporphyrin<sup>89</sup> was detected for any of the 4 sheep treated with Def-GaPP.



**Figure10B.** As a comparison: HPLC chromatogram of plasma sample spiked with GaPP (final concentration 0.2  $\mu\text{g/ml}$ ).



## **Manufacture/Chitogel Preparation:**

The gel consists of 3 parts: chitosan, dextran and a buffer solution. Currently, Def is dissolved in the buffer that is used to dissolve the dextran aldehyde before it is mixed with Chitosan to form a gel. This means this is free drug in gel.

- a. To prepare the Chitogel, 300 mg dextran-aldehyde, 10 ml succinyl-chitosan, and 10 ml of a buffer solution is mixed in situ in the operation theatre. The buffer solution is either drug free or contains Def-GaPP.
- b. Def and GaPP are individually dissolved in buffer, making a Def-buffer solution and a GaPP-buffer solution. Both of these solutions are able to be mixed with the dextran powder to create a solution to be mixed with the Chitosan. Depending on what combination is required these solutions can make up a Def Chitogel, GaPP Chitogel or a Def-GaPP Chitogel.

Def is water soluble and dissolves in the buffer over 1 day. GaPP is low water soluble and dissolves in the buffer over 3-5 days. Both buffer solutions are stored at room temperature because storing in the fridge causes precipitation of the drugs, which decreases their antibacterial activity. To prevent drug degradation, stock solutions are also stored in the dark. Buffer solutions are used within 2 weeks.

Stability: The drug concentrations of a Def- and a GaPP-buffer solution (freshly prepared and after 3 weeks of storage at room temperature, protected from light) were tested by high

performance liquid chromatography (HPLC). No loss of drug was observed and there were no degradation products detected.

**Sterility:** Once the drugs are dissolved in buffer, the buffer will be filter sterilised in an aseptic environment using 0.22-micron sterile filters. Following the sterilisation process, the integrity of the filters were confirmed by the Bubble-Point Test (quality control 1). An aliquot of each buffer (100 ul) will be streaked out on an agar plate and incubated at 37°C for 24 hours to confirm the absence of bacteria (quality control 2). In case of bacterial contamination, the buffer was again filter sterilised and tested for bacteria. Sterile Def-buffer and GaPP-buffer will be stored at room temperature protected from light and used within 2 weeks; the unsterile buffer discarded.

## 2 Wound healing in chronic rhinosinusitis

### 2.1.1 Definition and Disease Burden

Chronic rhinosinusitis (CRS) is a disorder characterized by mucosal inflammation of the nose and paranasal sinuses lasting at least 12 consecutive weeks. There are four pairs of paranasal sinuses which along with the nasal cavity may be affected by CRS<sup>98</sup>. These are pneumatic or air-filled extensions of the respiratory part of the nasal cavity, they are lined by pseudostratified columnar ciliated (respiratory) epithelium and are named according to the bones in which they are located, namely the frontal, ethmoid, sphenoid and maxillary sinuses. Multiple extrinsic and intrinsic etiological factors play a role, in combination, to cause prolonged symptoms and signs associated with this condition. The Task Force on Rhinosinusitis, established in 1996<sup>99</sup>, passed the first consensus to subclassify rhinosinusitis into acute and chronic based on the duration of symptoms:

- 1) < 4 weeks – Acute Rhinosinusitis
- 2) 4-12 weeks – Subacute Rhinosinusitis
- 3) > 12 weeks – Chronic Rhinosinusitis

The diagnosis of chronic sinusitis is based on the presence of major and minor clinical symptoms (Table 4.1) that persists more than 12 weeks along with objective evidence of inflammation by endoscopy and/or CT scan found in the sinuses<sup>100</sup>. This is usually associated with the presence or absence of polyps<sup>98</sup>, hence, further phenotypically classified as CRS with nasal polyps (CRSwNP) or CRS with absence of nasal polyps (CRSSNP). The European position paper on Rhinosinusitis and Nasal polyps 2020<sup>101</sup> has further simplified it and defined as defined as: presence of two or more symptoms, one of which should be either nasal blockage / obstruction / congestion or nasal discharge (anterior / posterior nasal drip): 1. facial pain/pressure; 2. reduction or loss of smell; for  $\geq 12$  weeks; with validation by telephone or interview.

**Table 4. 1: Symptoms of sinusitis**

| <b>Major Symptoms</b>                         | <b>Minor Symptoms</b>      |
|-----------------------------------------------|----------------------------|
| <b>Nasal obstruction/blockage</b>             | Ear pain/pressure/fullness |
| <b>Nasal discharge/purulence/ discoloured</b> | Headache                   |
| <b>Facial pain/pressure</b>                   | Halitosis                  |
| <b>Facial congestion/fullness</b>             | Fever (all nonacute)       |
| <b>Fever (acute rhinosinusitis only)</b>      | Fatigue                    |
| <b>Nasal discharge / postnasal drainage</b>   | Dental pain                |
| <b>Hyposmia/anosmia</b>                       | Cough                      |

### 2.1.2 Epidemiology:

CRS affects 10-20% of the western population<sup>102,103</sup>, causing a significant impact on quality of life of the individual and significant socio-economic burden on the community. The prevalence of CRS varies according to geographical region and is influenced by the methods used for diagnosis. Hence, in the USA is reported to be between 12.5-16%<sup>104</sup> and is listed as the second most common chronic disease after orthopaedic impairments, and is more common than ischaemic heart disease and hypertension. In Europe, the prevalence ranges from 6.9%-27.9%<sup>103</sup> and in Korea of 6.95%<sup>105</sup>. Sinusitis is one of the most common primary care presentations in Australia, with 1.4 in every 100 general practice outpatient visits estimated to be for rhinosinusitis<sup>102</sup>. In 2014-15, 7.1 million Australians were diagnosed to have a chronic respiratory condition by the National Health Survey (NHS) which includes a host of sinus related illnesses, 2.5 million with Asthma, 4.5 million with allergic rhinitis and 1.9 million people with chronic sinusitis<sup>106</sup>.

CRS contributes to a significant amount of healthcare expenditure due to direct costs arising from physician visits and medical treatment. Studies have reported that patients diagnosed

with CRS undergo an additional 3.5 outpatient visits , requiring 5.5 prescriptions more than non-CRS patients, and also suffer excessive financial costs of \$773 +/- 300 per year<sup>107</sup>. The actual economic burden to society is significantly greater when calculated along with the loss of productivity and absenteeism from work for health-related reasons. In the working age population, an estimated average loss of 4.8-5.7 days' work in a week compared to healthy people is due to CRS<sup>107</sup>. CRS also impacts a patient's quality of life significantly and comparative studies find lower quality of life scores in CRS patients when compared to congestive heart failure, angina, chronic obstructive pulmonary disease or back pain.

### **2.1.3 Patho-physiology**

Factors contributing towards CRS have been broadly categorized into intrinsic (host related factors) and extrinsic (non-host related factors). Intrinsic factors predispose the patients to the development of CRS, such as anatomic / structural abnormalities, genetic diseases and immune mediated abnormalities. Anatomic variations narrow the sinus drainage pathways in the frontal recess and osteomeatal complex and any inflammation in these areas lead to complete sinus obstruction, mucus stagnation and bacterial colonization and superinfection<sup>108</sup>. If associated with underlying genetic disorders such cystic fibrosis<sup>109</sup> and primary ciliary dyskinesia reduce ciliary movement and mucociliary clearance, this further contributes to mucus stasis and bacterial colonisation<sup>110</sup>. Non-infectious causes commonly seen associated with CRS are allergy and gastro-oesophageal reflux disease<sup>111</sup>, although their causative mechanism remains debated. Other intrinsic factors such as defects in innate immune responses and/or the physical barrier can also contribute to the development of CRS when exposed to pathogens including microbes<sup>112</sup>.

Extrinsic factors such as microbial agents, cigarette smoke and environmental irritants can initiate inflammation in CRS. Use of tobacco is one of the common extrinsic factors associated with CRS, tobacco smoke on epithelial cells downregulates the cystic fibrosis transmembrane conductance regulator (CFTR) gene and thereby contributes to the formation of thick mucus, facilitating the growth of bacteria<sup>113</sup>. Studies have reported that tobacco smoke may enhance biofilm formation<sup>114</sup>. Animal studies have shown that environmental irritants such as air pollution can also cause structural damage to the ciliated epithelium and affect the mucociliary clearance<sup>115</sup>. A recent Canadian study also suggests that certain pollutants may predispose to polyp formation<sup>116</sup>.

CRS patients with medically and surgically recalcitrant disease have characteristic features of chronic inflammation with persistent infection which are often attributable to the presence of biofilms<sup>117,118</sup>. Biofilms are found in 25-100% of CRS patients<sup>117</sup>, with *S. aureus* cultured in approximately 50% of them<sup>118</sup>, common organisms isolated from the adult CRS population include aerobes such as coagulase negative *Staphylococcus* (35%), *Corynebacterium* spp (23%) and *Staphylococcus aureus* (8%) gram-negative bacteria and anaerobic<sup>119</sup> organisms. They include *Prevotella*, *Fusobacterium*, *Pepto streptococcus* and *Propionibacterium*. In children *Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis*<sup>120</sup> are seen both in CRS and during episodes of acute exacerbations.

Among the bacterial causes *Staphylococcus aureus* is the most common pathogen isolated by culture in both CRSwNP and CRSsNP<sup>121</sup>. Superantigens produced from bacteria such as *S. aureus* have been thought to play a pathogenic role in CRS. These antigens bypass antigen recognition and promote polyclonal T lymphocyte proliferation with massive release of cytokines which is very often seen in the case of polyp production<sup>122</sup>. Compared to free floating planktonic bacteria, those living as communities (biofilms)<sup>123</sup> contained within extracellular polymeric substances (EPS) have longer survival and propagation rates. Biofilm

bacterial communities are complex and coexist with different strains and species. Cryer et al in 2004 was the first to provide evidence of biofilms on sinus mucosa<sup>124</sup>. Slow growing persister bacteria within biofilms resist conventional antimicrobial treatment leading to chronicity and disease recalcitrance.<sup>125</sup> Psaltis et al found that biofilms are associated with recalcitrance of disease especially in the post-operative period after endoscopic sinus surgery<sup>126</sup>. The presence of biofilms in CRS and absence in controls is a common finding in the literature<sup>127</sup>, however the mechanism(s) by which they trigger and perpetuate inflammation are still unanswered and warrant further research.

The role of fungal infections in the pathogenesis of CRS has been debated for decades. They are broadly grouped into invasive and non-invasive fungal infections. The fungal hypothesis suggests that the presence of fungal elements in CRS activates T cells and eosinophils causing inflammation and damage to mucosa<sup>128</sup>. However, this theory was disproven when fungal elements were seen in control patients and antifungal treatments were ineffective to treat CRS patients<sup>129</sup>. In the absence of clinical improvement with antifungal therapy, the role of fungi in CRS is circumstantial, but studies have indicated that they may have a synergistic action with bacteria<sup>130</sup>.

Viral infections of the upper respiratory tract play an important role in the etiopathogenesis of acute sinusitis rather than in CRS, however, the presence of virus in CRS patients has been shown to be associated with worse disease severity<sup>131</sup>, this may be in part due to an abnormal or exaggerated immune response reported in CRS patients with viral positivity<sup>131,132</sup>.

### 2.1.4 Management of recalcitrant CRS

Diagnosis of CRS requires documented presence of two or more symptoms, along with objective evidence of inflammation either on endoscopy and/or on CT scan. Patient symptoms are documented on a widely accepted validated questionnaires such as Sino-Nasal Outcome Test 22 (SNOT – 22)<sup>98</sup>. This patient-reported outcome measures enables the physician to assess the severity of disease based on a numerical score and also provides a tool to monitor patient response to treatment. The Adelaide Disease Severity Score (ADSS) is another scoring system that has been validated against the SNOT 22 and shown to have higher correlation with Lund–Mackay CT score and the Lund–Kennedy endoscopy score<sup>133</sup>. Unlike the SNOT22, this scoring system only assesses the severity of the 5 most common rhino logic symptoms. Another instrument used in rhinology to quantify symptom severity is the visual analogue scale (VAS)<sup>134</sup>. In this scale, patients graphically represent the severity of their symptoms on a continuous scale ranging from 1-10.

The tools used to document objective evidence of CRS are the Lund-Mackay and the Lund-Kennedy Score (LKS) scores. The Lund-Mackay CT scan staging system grades the opacification of the sinuses. Although commonly used it has never been truly validated. It is however an easy and a reproducible method to assess treatment response in CRS<sup>135</sup>. The LKS is an endoscopic scoring system that assesses the severity of disease based on the presence and severity of secretions, mucosal oedema, scarring and crusting. The Modified LKS system, proposed by Psaltis et al, is a refinement of the LKS, whereby scarring and crusting are removed from the scores. This allows the system to be used also for un-operated patients and removed the effect that poor surgical technique, often associated with crusting and scarring, may have on the score. Psaltis et al found higher intra and inter-rater reliability of the MLKS compared to the LKS and a higher correlation to symptom scores<sup>136</sup>.



There are currently (at least) 2 challenges in the management of RCRS, these are:

- (1) biofilms that have up to 1000 times the resistance to routine antibiotics when compared with planktonic bacteria<sup>137</sup>, and are therefore difficult to gain control of with current antimicrobial therapies and
- (2) the anatomy and orientation of the sino nasal cavities make it a difficult-to-access area for the delivery of therapeutic agents.

## 2.1.5 Medical Management

The Management of CRS has been directed predominantly against microbes that cause inflammation and a combination of local and systemic therapies against other causative factors of inflammation along with those that bring symptomatic relief. They could be generally classified into:

1. Local therapy – Saline Irrigation,

Steroid Irrigation, Antibiotic irrigation & Antifungal application

2. Systemic therapy – Antibiotics, Steroids, Anti Leukotriene, Anti-Ig E, Immunotherapy (anti IL-5 Dupilumab) and aspirin desensitization's.

### Local Therapy:

**a. Saline:** The primary role of saline irrigation is removal of secretions/mucous with inflammatory substances in the sinuses. High-volume (>100ml) saline irrigations are a well-accepted and widely used cost-effective treatment for all forms of CRS. The use of Saline along with antibiotics and/or adjunctive treatment is supported by A-1 evidence and is a recommended form of treatment by the comprehensive international consensus statement<sup>138,139</sup>. Low-volume delivery either by spray topical or nebulization is generally not

recommended because it has been shown to be less efficacious<sup>140</sup>. Studies examining different formulations of saline irrigations have found similar effects with both Isotonic and hypertonic saline, except with a higher rate of minor side effects while using hypertonic solution<sup>141</sup>.

**b. Intranasal corticosteroids (INCS):** Evidence based Cochrane review of Randomised controlled trial (RCT's) show an improvement in symptom and endoscopic disease severity scores in both CRSsNP and CRSwNP patients. There is a definite reduction in the polyp size and associated improvement in olfaction<sup>140</sup>. Various types of INCS are in use such as beclomethasone propionate, mometasone furoate, fluticasone propionate and budesonide which have similar effects<sup>140</sup>. Early concerns of systemic absorption and its impact on the hypothalamus-pituitary-adrenal axis is now settled with very minimal side effects<sup>142</sup>. Hence, this is often used as the first line of therapy.

**c. Saline Irrigation with Corticosteroids:**

Topical delivery of gluco-corticosteroids in combination with saline irrigation has gained increased popularity amongst clinicians despite its off-label use. RCTs have demonstrated the efficacy of controlling the disease with significant improvements in validated symptom and endoscopic scores of disease severity, particularly in the post-operative setting<sup>143</sup>. Although safety studies suggest that steroid irrigations are generally well tolerated with little in the way of side effects on hypothalamus-pituitary-adrenal (HPA) axis suppression, a study by Soudry et al<sup>144</sup> did show mild but reversible asymptomatic HPA suppression in a small subset of patients, all of whom were concurrently using inhaled corticosteroids<sup>145</sup>. Early evidence suggests that high-volume corticosteroid irrigations (i.e., techniques that involve delivering >100 mL of solution into the nasal cavity along with budesonide)<sup>146</sup> irrigations are more effective than low-volume corticosteroid spray techniques (i.e., meter-dosed spray, atomized,

or nebulized solutions)<sup>146</sup>, clinical trials are required before a recommendation on optimal delivery method can be provided.

#### **d. Saline Irrigation with Antibiotics or Antifungal:**

Systematic reviews<sup>147-150</sup> have shown improved short-term symptom score and no difference in sinus specific quality of life (QOL) with use of topical antibiotics for CRSsNP. Bardy et al demonstrated this using a high-volume (240 mL divided between 2 nasal cavities) mupirocin irrigation compared with placebo in a specific cohort of patients with a sinus culture positive for *Staphylococcus aureus*<sup>151</sup>.

Use of topical amphotericin B demonstrated no benefit compared with placebo for patients without nasal polyps in randomized control trials (RCT's)<sup>129</sup>. Rudmik et al<sup>152</sup> reviewed the use of topical therapies in CRS and recommended the use of irrigations with saline and nasal steroid treatment instead of the use of topical antimicrobials and antifungal treatments. Therefore, use of topical antifungals for chronic sinusitis without nasal polyps is not a routine practice.

#### **Systemic Therapy:**

**a. Oral corticosteroids:** Steroids as anti-inflammatory agents are now the accepted line of therapy and is widely accepted<sup>153</sup>. A combination of oral and topical steroid therapy are followed to reduce polyp size more efficiently<sup>154</sup>. No more than 2–3 courses per year is suggested to avoid risk of side-effects due to high dose or frequent use<sup>155</sup>. Although high-level evidence exists supporting oral steroid use in CRSWNP patients, evidence supporting their use in CRSsNP is lacking<sup>138</sup> and therefore they are not routinely recommended in these patients, except for those of fungal aetiology.

**b. Antibiotics:** CRS remains one of the most common indications for which oral antibiotics are prescribed, but, evidence supporting their use in the treatment of CRS remains limited<sup>156</sup>.

A Cochrane review assessing the value of antibiotics for CRS, revealed a limited overall benefit and a transient improvement in QOL was seen in some CRS patients receiving 3 months of macrolide therapy<sup>157</sup>.

A recent systematic review of published low-dose macrolide studies suggested that this antibiotics class may be more beneficial in CRSsNP than CRSwNP patients, with subgroup analysis showing more benefit when used for 24 weeks compared to shorter durations. No difference between 14-membered and 15-membered ring macrolides was noted<sup>158</sup>. The effect of low-dose macrolides is thought to be primarily mediated through their local immune modulating/anti-inflammatory properties rather than their antibiotic action<sup>159</sup>. Although macrolides are generally well tolerated with a favourable side-effect profile, the FDA has released a recent warning of the possible increased risk of all-cause mortality over 10 years in patients with cardiac disease taking macrolides<sup>160</sup> suggesting caution in these patients. Given the lack of evidence supporting the use of non-macrolide oral antibiotics for CRS and the risk of serious adverse effects and emerging issues regarding antibiotic resistance, the use of these agents should be justified by endoscopic evidence of an infective exacerbation or complication. In such conditions, antibiotic therapy should ideally be culture directed. Topical antibiotics may seem to have a better systemic side effect profile than their oral counterparts, they are associated with an increased local side effects such as burning, bleeding, and nasal dryness and to date, no high-level evidence supports their use for CRS.

**c. Antifungal Therapy:** The role of antifungal therapy for CRS in general remains controversial, Cochrane review of this topic published in 2018 included eight studies with 490 adults. Results indicate a lack of high-level evidence to support the use of oral or topical

antifungal agents<sup>161</sup>. As a result there are no generally recommended consensus guidelines for the routine use of these agents in treatment of CRS<sup>162</sup>. But in case of culture proven invasive fungal aetiology, systemic and local therapy is recommended<sup>163</sup>. The use of antifungals is being recognized as ineffective in the treatment of CRS, as reported by a meta-analysis that included six studies (380 participants). The use of topical antifungals was investigated in five studies and one study investigated the effect of systemic antifungals. This pooled meta-analysis demonstrated that there was no significant benefit of topical or systemic antifungals over the use of placebo for any of the outcomes.<sup>164</sup>

#### **d. Monoclonal antibody:**

Strong patho-physiological link between nasal polyp and Asthma has led to research in the use of biological therapy in CRS<sup>165</sup>, trials of monoclonal antibodies with FDA approval as chronic inflammatory conditions are currently under investigation for CRS. Independent phase 3 study in CRS patients receiving subcutaneous injections of Omalizumab, an anti-Ig E antibody approved for patients with refractory asthma, has shown improvements in polyp sizes, nasal and asthma symptoms, there was also improvements in radiological severity scores and QOL with 16 and 24 weeks of treatment<sup>166,167</sup>. These agents act by targeting the IL-5 pathway, which is thought to be central for Th2 eosinophilic inflammation associated with CRS. Erlizumab and Mepolizumab, humanized monoclonal antibodies approved for the treatment of severe asthma, both showed a significant reduction in polyp size in patients treated for 4 weeks compared to placebo<sup>168,169</sup>. Dupilumab a fully human monoclonal antibody that inhibits signalling of interleukin (IL)-4 and IL-13, key mediators of type 2 inflammation. Currently, it is FDA approved for use in atopic dermatitis, asthma and CRS. Results from two multicentred, randomized, double-blind, placebo-controlled, parallel-group phase 3 trials involving 276 patients showed that adult patients with severe CRS there was

reduced polyp size, sinus opacification and severity of sinus symptoms. The medication was generally well tolerated by patients with a few experiencing a slightly high rate of headaches, epistaxis, injection site reactions nasopharyngitis, and worsening of nasal polyps and asthma than placebo<sup>170</sup>. Biologics hold promise as a means of offering patients personalized endotype-driven therapy, but their high cost remain important challenge to be recommended for routine care<sup>171,172</sup>.

### **Challenges and current research in Medical Therapy:**

CRS is the most common diagnosis treated with antibiotics<sup>173</sup>, with repeated use its effectiveness has decreased leading to increasing levels of antibiotic resistance<sup>174</sup>. Treatment of CRS with antibiotics are reserved only for those patients with evidence of bacterial infection manifesting as fever, purulent discharge and pain or pressure<sup>175</sup>. Those who fail to respond to antibiotic treatment have usually received multiple courses of antibiotics before going in for surgery, this includes both systemic antibiotics and topical corticosteroids<sup>176</sup>. Increasing treatment cost due to repeated use of antibiotics to the tune of direct cost of \$ 921 USD per patient year and yearly economic cost of \$1539 USD per patient has been reported by Bhattacharya et al in a cohort of 332 CRS. They also found an average 2.7 antibiotic course for a duration of 18.3 weeks, over a 12-month period.

Globally there is a rise in antibiotic resistance and a reduction in the number of novel antibiotics in clinical development, new experimental treatments are being developed. Since the use of antimicrobials in CRS remains contentious, anti-inflammatory drugs have become the mainstay of treatment for CRS. Intranasal corticosteroids remains effective in both

CRSsNP and CRSwNP patients and are recommended based on evidence-based review<sup>177</sup>. The use of monoclonal antibodies (MAs) as biologic therapies are promising new therapies, these target inflammatory signalling molecules to treat inflammation in CRS<sup>178</sup>. Drilling et al<sup>179</sup> used bacteriophage (phage) for topical application in the treatment of CRS specifically in drug resistant clinical Isolates in sheep and found improved outcomes in comparison to saline. The use of phage as a single dose proved to significantly decrease the formation of biofilms of *Pseudomonas aeruginosa* in vitro<sup>180</sup>, it was shown to be safe and efficacious in an in vivo model when used as a cocktail (CT-PA) at a concentration of  $10^8$ - $10^{10}$  PFU/mall. Bacteriophage (phage) therapy as intranasal irrigation with the phage cocktail AB-SA01 of doses  $3 \times 10^9$  plaque-forming units (PFU) for 14 days was proven efficacious in the treatment of patients with recalcitrant CRS due to *S aureus*<sup>181</sup>. The challenges in antibacterial therapy in CRS has led investigators to look into alternative treatments such as replacement of abnormal microbiota<sup>182</sup>, the principle was using healthy commensal bacteria *Staphylococcus epidermidis* (SE) in a mouse sinusitis model supporting microbial rehabilitation as a promising avenue for the management of CRS.

The overall outcome of the current medical treatment protocols is limited in CRS; hence they require surgical clearance of obstruction to remove diseased mucosa and bony deformities. Thereby, post-surgical delivery of anti-inflammatory and anti-microbial drugs to the mucosal sinuses deep within crevices could be done to eradicate biofilms and microbial colonies to achieve long term benefit.

### 2.1.6 Surgical Management

Surgical treatment of CRS has come full circle in its primary principle and technique<sup>183</sup> with the invention of Microscopes and endoscopes, along with understanding in the physiology of

the nasal mucosa based on the work of Messerklinger, a radical shift to the middle meatus as the route of approach to remove focused diseased mucosa and in restoring the drainage of the sinuses is the standard of care. Mucosal preservation being the corner stone for the mucociliary clearance of sinus surgery and hence the term Functional endoscopic Sinus Surgery<sup>184</sup>.

### **2.1.7 Indications and aim of ESS**

Surgery as a treatment of CRS is indicted only in those patients in whom maximal medical treatment fails or there are anatomical or structural abnormalities that prevent the resolution through medical therapy. However, this list of indication has changed with the improvement of instruments and surgical technology to include vasomotor rhinitis, Septal deviations, turbinate surgeries, polyps in the nares or antrum of sinuses, mucocèles, retention cyst, dacrocystic pathology epistaxis that is refractory to routine treatment<sup>185</sup>.

The aim of surgery itself is trifold<sup>186</sup>:

- (1) to improve aeration of the sinuses
- (2) to reduce the inflammatory burden of disease
- (3) to increase the access of the sinuses

### **2.1.8 Outcomes of ESS<sup>187</sup>**

ESS as a treatment of choice for CRS has been effective both with and without polyps and has been well evaluated and statistically proven by multiple studies in treatment of chronic rhinosinusitis refractory to medical management<sup>188-192</sup>. A systematic review and meta-analysis demonstrates, improvement in patients having reduction in use of antibiotics, oral



steroids, inhaler use and improved asthma controls with decrease in symptoms<sup>193</sup>. Even in CRS patients with immunodeficiency, ESS is better in terms of outcomes in compared to Medical therapy<sup>194</sup>.

Although surgical treatment brings relief in majority of patients, a proportion of them approximately 16% required revision surgery because of pre-existent risk factors or etio-pathological factors such as Aspirin exacerbated respiratory disease, allergic fungal sinusitis or a prior polypectomy<sup>195</sup>. ESS impacts the patient with a considerable amount of humanistic and economic burden which is also part of the outcome in terms of expenditure for gaining relief<sup>196</sup>, and this significantly affects their quality of life (QoL)<sup>197</sup>

### **2.1.9 Complications of ESS**

With a rise in the number of surgeries performed each year so are the rising cost of complications and undesired outcomes of surgery. Complications after ESS are classified as Major and Minor.

#### **Major Complications**

Major complications include intra-operative injury to orbital contents or orbital haemorrhage causing vision loss or diplopia, skull base penetration causing CSF leak with the associated risk of meningitis or injury to major vascular structures including the anterior ethmoid artery or carotid artery<sup>198</sup>. Early reports suggest that the risk of major complications could be as high as 1-4%, but recent studies have shown that the rate is in a range of 0.36%-0.46% for primary ESS and revision ESS, due to improvements in anatomical knowledge, surgical training, equipment and the use of intra-operative navigation in patients undergoing ESS including Skull base surgery<sup>199</sup>.

## Minor Complications

The incidence of these is more common and frequent. Minor complications include bleeding, infection and synechiae formation with the later having a reported incidence in the range of 15-30%. Synechiae and adhesions may lead to stenosis of the sinus openings that are widened during ESS which interfere with normal muco-ciliary transport and mucosal function of the sinuses. This among many other factors lead to recurrence of disease and treatment resistance.

### 2.1.10 Sino-nasal wound healing

The sino-nasal mucosa is a defensive blanket that spreads from the beginning of the airway in the nose and continues into the lungs. It forms a physical barrier and also warms and humidifies the inspired air before it reaches the lower air way<sup>200</sup>. The epithelium is a pseudo stratified layer of columnar cells with and without cilia interspersed with goblet and basal cells which produce mucous and maintain the integrity of the barrier. The epithelium is supported by a basement membrane situated on the lamina propria, which is formed by superficial sero mucinous layer and a deeper vascular layer. Lymphoid tissues consisting of plasma cells and lymphocytes are present under this which help in protection at the barrier. Sino nasal wound healing has been studied in animal and human models and it has been an established fact that preservation of the basement membrane is critical for optimal wound healing, possibly due to the location of stem cells in this region of the epithelium.

Animal Model studies: Knowlton<sup>201,202</sup> and Hilding<sup>203,204</sup> are the pioneers in the study of sino-nasal healing in rabbit and dog model in the early part of 20<sup>th</sup> century. In their studies

they showed that the regenerated epithelium after an injury demonstrated a variety of response including acute and chronic inflammation, ulceration or granulations and fibrosis. They observed that the subepithelial tissue was replaced by dense connective tissue in the lamina propria devoid of glands. Striping of periosteum lead to new bone formation with bone remodelling and polyp formation in the regenerated mucosa<sup>205</sup>. They also observed that preservation of the basement membrane enabled regeneration of epithelium within 3 days however damage to this layer resulted in delayed and disorganized wound healing<sup>206</sup> with long term effects on the mucociliary action.

Human Model studies: Sino-nasal wound healing has been studied in humans much more rigorously since the advent of endoscopes which enable a more magnified view of the mucosa,<sup>207,208</sup> Four overlapping stages of healing have been described post operatively. These stages correspond to stage 1 (7-12 days post injury) which is characterized by blood clot/crust covering the wound. Stage 2 (Week 2-4) is typically characterised by granulation formation while in Stage 3, oedema sets. It is not until stage 4( usually 12-18 weeks post-operatively) that macroscopic normalization is seen.

Patho-physiology of Adhesion Formation: As described earlier, one of the complications of sino-nasal wound healing is adhesion formation. Adhesions result from epithelial and fibrin migration along crusts/clots connecting two traumatized surfaces. Studies suggest that CRS induces greater expression of TGF- $\beta$ <sup>209</sup>, leading to greater fibrosis formation and ostial stenosis. 15-30 % of patients undergoing ESS develop adhesions and this is reported to be the commonest complication seen following sinus surgery. Adhesions may lead to muco-ciliary dysfunction and may obstruct sinus ostium<sup>210,211</sup>, and have been estimated to the

primary causative factor of disease recurrence in up to 60% of patients needing revision surgery<sup>212,213</sup>.

Adhesions around the ostium causing stenosis are in the order of 59.5% in the frontal ostium and 25% in the remaining sinuses<sup>214,215</sup>.

### **2.1.11 Adjuncts for improved wound healing in CRS**

Minimal mucosal damage and excellent haemostasis are the foundation of FESS and will also improve wound healing. These are highly variable and can be affected by the skills and level of experience of the surgeon and the field provided by the anaesthetist. Post-operative measures including irrigations and meticulous debridement can also improve wound healing.

Per operative considerations :

Nasal Packing: Traditionally a wide variety of packing materials have been used to act as a tamponade to minimize post-operative bleeding. Materials used include ribbon gauze impregnated with a lubricant (e.g., Vaseline Ribbon Gauze and Claudin Nasal Ribbon Gauze, Lohmann Corp, Hebron, Ky) and antibiotic ointment (e.g., Bismuth Iodoform Paraffin Pack, Evans Medical Ltd, Leatherhead, United Kingdom)<sup>216,217</sup>. Gauze packing although very effective in obtaining haemostasis confer significant discomfort to the patient when in situ but allows significant ingrowth of fibrinous tissue into the gauze which can result in bleeding upon its removal. This itself can predispose to adhesion formation and mucosal dysfunction. To address this, absorbent nonadherent biomaterial nasal packings have been developed and include Haemostats and Antiadhesive agents of animal and plant origin<sup>218</sup>. Among the haemostats used intraoperatively porcine gelatine and thrombin combination (Surgiflo, Ethicon Inc, Somerville, New Jersey); topical anti-fibrinolytics such as epsilon-aminocaproic acid (Amicar, Ederle Parenterals Inc, Carolina, Puerto Rico) and tranexamic acid (Cyclopoid,

Pfizer, Puurs, Belgium); and hyaluronic acid , Oxidized regenerated cellulose (Surgicel Nu-knit; Ethicon Inc.), Fibrin glue (Quixil; Omrix Co., Brussels, Belgium), Microporous polysaccharide hemosphere (MPH; Medafor Inc., Minneapolis, Minnesota, USA) and FloSeal (Baxter International Inc., Deerfield, Illinois, USA) have all been investigated in human studies and used for their intraoperative haemostatic properties after ESS. Anti-adhesive biomaterials such as MeroGel (Medtronic Xomed, Jacksonville, Florida) a hyaluronic acid with absorbable starch, Carboxy-methylcellulose (CMC; AthroCare, Glenfield, UK) a plant-based biomaterial is all used. Unfortunately, patients still report significant discomfort with these packing materials causing varying amount of adhesion which is undesirable for good wound healing outcome<sup>29</sup>.

Other complications of Nasal Packing include foreign body granulation, dislodgement, septal perforation, aspiration, toxic shock syndrome, obstructive sleep apnoea and even death <sup>219,220</sup>. Hence, research for a better nasal packing biomaterial that is least traumatic to the mucosa with properties that enhance mucosal healing is much needed.

### **Post-Operative Care considerations in wound healing:**

Saline irrigations are commonly used post-operatively. Irrigations aim to improve mucociliary clearance, remove crusts, pus and debris, and thereby improve mucosal healing.

Endoscopic cleaning/debridement: Post-operative endoscopic cleaning or debridement is removal of dried mucus crusting and clots that can act as bridges for epithelial and fibrin migration between traumatized surfaces. Surgeons in general consider this as crucial as the surgery itself and when done at right time forms a critical post-operative care for an optimal outcome. At present there is no consensus on the ideal time or frequency of debridement

post-FESS<sup>221</sup>, but it is generally agreed that the first debridement should occur within 10-14 days of surgery to minimize the chance of adhesion formation<sup>211,222,223</sup>. An RCT conducted by Bugten et al<sup>210,224</sup> found that there was significant reduction of adhesion formation in patients undergoing post-operative debridement at day 6 and day 12 in comparison to those not debrided. Whereas they also found that debridement increased pain needing more pain relief medication and prolonged treatment. Although, it was found that increased crusting caused significantly increased adhesions supporting the theory that uncleared debris along with bony fragment forms potentially a bridge for fibrotic activity and bacterial growth. Rudmik and Kennedy et al in their systematic review of level 1 RCT recommended the use of nasal saline irrigation, sinus cavity debridement, and standard topical nasal steroid spray early in the postoperative care<sup>225</sup>. Hence, the current evidence suggests early post-operative cleaning, in the first week and the optimal frequency of subsequent sinus cavity debridements was at 12 days - 2 weeks intervals<sup>226</sup>. During this procedure epithelial avulsion is a possibility as described by Kuhnel et al, who found 23% of his patients suffered mucosal avulsion if cleaned within 2 wks post operatively<sup>227</sup>. But, Kemppainen demonstrated that patients who received 3 sinus cavity debridement's within the first week after ESS had reduced nasal discharge scores compared to patients who received a debridement at 1-week after ESS<sup>228</sup> contrary to the earlier.

**Antibiotics:** Role of antibiotic in CRS and post-operative wound healing has been contentious, and there are no level 1 evidence studies to suggest routine prophylactic antibiotic prescription<sup>229</sup>. Saleh et al<sup>230</sup> conducted a meta-analysis of 3 trials demonstrating that the routine postoperative antibiotic prophylaxis did not show any statistically significant reduction in the incidence of infection, endoscopic scores, and symptoms. Antibiotics not only have direct antimicrobial effects that promote wound healing, many antibiotics also have

immunomodulatory functions and have been shown to affect ROS (reactive oxygen species) production an important signalling molecule in cell migration and wound healing<sup>231</sup>. Of the routinely used first line and second line antibiotics in CRS, Fluroquinolones such as Ciprofloxacin exerts anti-inflammatory effects in *S. aureus* Newman driven nasal inflammation<sup>232</sup>. Doxycycline promotes and hasten wound by means of its immunomodulatory and anti-inflammatory actions<sup>233</sup>, Gouzos et al<sup>231</sup>, have demonstrated invitro that Amoxicillin/clavulanate, commonly preferred antibiotic in rhinology for its activity against a majority of common nasal pathogen, does not have any effect on wound healing but other commonly used antibiotics such as clarithromycin, linezolid, and mupirocin have significant effects on ROS and promotes wound healing.

Anecdotally some surgeons recommend antibiotics if signs of infection are found during surgery or also if culture proven with sensitivity specific antibiotic. Although, some authors randomised patients to receive prophylactic antibiotics and found no difference in their subjective or objective outcomes after surgery<sup>234</sup> and others randomised patients to receive post-operative antibiotics and found no difference in either patient symptoms or bacterial growth culture<sup>235</sup>.

**Cortico-steroids:** The anti-inflammatory properties of steroids make these medications a cornerstone in the management of CRSwNP. Pundir et al<sup>236</sup> in a systematic review and metanalysis recommend pre-operative use of local and/or systemic corticosteroids in ESS for reduction in blood loss, operative time and improved surgical field quality. Systematic review of RCT by Rudmik et al<sup>225</sup> for early post-operative care, recommends the use of oral steroids with caution and to balance the local benefits with systemic side effects of oral steroids. A recent review by Poetker et al strongly recommends a short course of oral

corticosteroids for CRSwNP and those secondary to fungal aetiology post-operatively<sup>237</sup>. Rudmik et al also review looks at the use of intranasal topical steroid spray after surgery and found evidence for use to be level 1b quality and found maximum benefit in CRS patients with nasal polyps, by improving intra-operative surgical conditions and also in improving post-operative outcomes as it reduced the recurrence rate of polyps significantly<sup>222,223</sup>. Post-operative application of steroids in the form of irrigations or sprays have also become routine practice. High concentration intranasal dexamethasone application: ophthalmic drops (0.1%), prednisolone ophthalmic drops (1%), and ciprofloxacin/dexamethasone otic drops (0.3/0.1%) in revision ESS reduced the risk for sinus ostial stenosis and polyp recurrence<sup>238</sup>, this is non-standard & off-label use to deliver higher amounts and higher concentrations of topical steroid to the sino-nasal mucosa.

Intranasal Steroid sprays(INS) have become standard of care for their anti-inflammatory property and lengthens the time to recurrence of polyps significantly<sup>225</sup>. Multiple formulations are in use of which, Mometasone spray group had better healing scores, especially in nasal polyp<sup>239</sup> and Fluticasone propionate spray group had better symptoms as well as endoscopic scores at 5 years<sup>234</sup> and long-term benefit in reducing oedema and polyps with good safety profile. A RCT study by Rowe Jones et al has also shown that patients receiving intra nasal cortico-steroids also have a lower post-operative oral corticosteroid requirement<sup>234</sup>.

Intranasal saline douching using high volume dispensers with steroid solution such as budesonide(off-label) have also been used as wound healing adjuncts, topical irrigation demonstrated no significant adrenal suppression by Bhalla et al<sup>240</sup>, and Welch et al<sup>241</sup> demonstrated that twice daily budesonide nasal irrigations (0.5 mg/2 mL in 240 mL saline) post-operatively did not alter the serum cortisol or 24-hour urine cortisol levels. Intranasal



drug eluting stents also act as spacers have potential benefit but they run a risk of inducing inflammation as a foreign material<sup>152</sup>.

All these adjuncts have potential to reduce scarring and thereby avoiding the stenosis of the ostium but carry the risk of systemic absorption through the disrupted sino-nasal barrier.

### **2.1.12 Research in Nasal Packing:**

Draw backs in the use of removable nasal packing materials have led to ongoing research and development of absorbable biomaterials.

Biomaterials and Adhesion Prevention

#### **Animal studies:**

Extensive research has been conducted to study the nature of disease progression and wound healing in animal models including the sheep, rabbit and mouse. Sheep models are thought to be ideal due to their size, ease to inspect the sinuses using an endoscope and their similar nasal mucosa to humans<sup>74,242</sup>. Multiple studies have been conducted creating a bacterial sinusitis inoculation by blocking the maxillary sinus ostia with merocele along with *Bacteroides fragilis*<sup>243-249</sup>. Merocele as a biomaterial was studied in a sheep model to look for rate of re-epithelization, total surface of cilia and the maturity of the cilia in packed sinuses Vs non packed<sup>250</sup> by McIntosh et al and found no significant changes, this was replicated using Merogel in a CRS model of sheep and found no significant differences in adhesion or the other histological features of cilia regeneration on standardised wound after 1, 2, 3 and 4 months post operatively<sup>251</sup>.

Chitogel as a biomaterial has also been investigated in a sheep model of CRS by Athanasiadis et al<sup>252</sup>, where 20 Sheep infested with nasal bot fly (causing eosinophilic sinusitis) underwent a standardised mucosal injury. And they were randomised to be treated with Chitogel, Spray gel(Polyethylene glycol) and control. The results showed a greater advantage in the Chitogel treated group in cilia re-epithelization and re-ciliation and cilia grade( $p<0.05$ )<sup>252</sup>. The sheep model was also used to study drug delivery by MeroGel as a drug delivery, Robinson et al<sup>253</sup> studied the role of Prednisolone loaded MeroGel Vs plain MeroGel and found no difference. Le et al conducted a RCT in 54 Sheep frontal sinuses to study antibiofilm drug combinations namely single mupirocin flush, 12-hourly mupirocin flushes for 5 days, Citric Acid Zwitterionic Surfactant (CAZS) via hydrodebrider, gallium nitrate, CAZS with gallium nitrate, CAZS with mupirocin, and saline regular flushes in comparison to no treatment as control in a sheep sinus. Biofilm model and Confocal LSM evaluation showed regular Mupirocin washes showed significant reduction of biofilms that was sustained up to day 8<sup>91</sup>. More recently Chitogel as a drug carrier has been studied in a Sheep sinusitis model to deliver Deferiprone (an Iron Chelator), in combination with GaPP as an anti-biofilm agent. Preliminary results suggest that CD with Def & GaPP are safe and good bio-film elimination profile as compared to no treatment.

Rabbits have also been used because of their well pneumatized sinus cavities and their similar anatomy and immunologic reaction with those of humans, making them a good animal model for studying biomaterials<sup>254</sup>.

### **Human Studies:**

Merocel removable packs have been compared with MeroGel(a hyaluronic acid-based gel acts as an extracellular matrix scaffold for wound healing) in a number of studies. A double blinded RCT by Miller et al<sup>255</sup> found an adhesion rate of 8% in both groups, while Vaiman

et al and Pomerantz et al<sup>256,257</sup> showed no evidence of adhesion formation. Bugten et al studied Merocel Vs no packing and found statistically significant adhesion with no packing in comparison to Merocel packing<sup>224</sup>. Franklin and Wright compared MeroGel with Non-absorbable nasal packing and showed a trend towards improved endoscopic score post-operatively, but this was not consistent at all time points of post op evaluation at 2wks, 1, 3 and 6 months<sup>258</sup>. Wormald et al<sup>259</sup> studied the effect of MeroGel on wound healing following ESS in a single blinded RCT in 42 patients in comparison to no treatment. Post op follow up 2, 4 & 8 wks showed no significant difference and had an incidence of adhesion of 16.7% and 19% in MeroGel Vs No packing at 8 wks.

Fibrin glue commercially available as Quixil (Omrix Co., Brussels, Belgium) contains human thrombin and fibrinogen in conjunction with amino acids and salts, is another product that has been studied by Vaiman et al<sup>256</sup>. They compared Quixil with Merocel prospectively in 158 nonrandomised pts and followed patients up for 1 month. They found no adhesions in 77 of their Quixil treated patients and only 1 in the 64 Merocel treated patients. Building on this study the authors conducted a randomised, double blinded study in 64 patients where Merocel was applied only for 2 days and all the patients were evaluated at 3 months post operatively. No adhesion was found in either group of patients, which is comparatively less than all other studies. This is probably due to difference the grading system used by the authors in comparison to others.

Floseal another commercially available fibrin glue was studied by Chandra et al<sup>260</sup> in a double blinded RCT in 20 patients in comparison to fibrin soaked Gelfoam. Patients were followed at 1 and 6 weeks post operatively. This study showed a significantly higher mean of adhesions in the floseal treated sinuses 56%, with increased granulation formation at 21 days

post op. Histopathological evaluation of the adhesion showed foreign body in the scar tissue suggesting incorporation of the Floseal. Similar results were noted by Shirme et al <sup>213</sup> when they used Floseal to stop bleeding in the middle meatus and compared to the untreated side in retrospective analysis of 172 pts, where the incidence of adhesion was statistically higher (18.9%) with medialisation of Middle turbinate in comparison to medialisation alone without floseal (6.7%). An interesting outcome of this study was the multivariate analysis of adhesions in comparison to the various surgical and demographic variables. They found incidence of adhesion was significantly higher with the use of Floseal which indirectly explains the role of coagulation in adhesion formation which is enhanced by floseal<sup>213</sup>. And in contrast, Jameson et al found no difference between Flo seal and No treatment in a double-blinded RCT up to 3 months of follow up.

Carboxy Methyl cellulose (CMC) is another nasal packing material that has been studied as a mesh and gel<sup>261</sup>, Kastl et al published data did not showed any significant difference clinically on wound healing significantly between the two <sup>262</sup>.

Gel film- denatured porcine collagen has also been studied in comparison to MeroGel. In Catalano and Roffman's<sup>263</sup> study of 115 pts after a minimally invasive sinus technique (MIST), Gel film was placed as a stent and followed up to 3 months. They observed a significant increase in adhesion in sinuses stented with Gel film compared unstented sinuses . Tom et al <sup>264</sup> also conducted a RCT comparing Gel film to no treatment with patients acting as their own controls and results showed no significant difference in adhesion, but there was increase granulation tissue formation on the Gel film side.

Mitomycin C a topically applied agent derived from *Streptomyces cespitosus* has also been shown to have inhibitory activity over nasal fibroblast by inducing apoptosis.<sup>265</sup>. Chung et al studied application of mitomycin on a cotton in the middle meatus compared to saline and followed up to 2 months. They observed relatively less adhesions in the mitomycin applied side although this did not achieve statistical significance<sup>266</sup>. Anand et al<sup>267</sup> and Chan et al<sup>268</sup> found similar out comes in double -blinded RCT conducted in different centres.

#### **2.1.12.1 Chitogel in CRS**

Chitosan a polysaccharide hydrogel prepared from chitin, a polymer that is found in a large number of natural animal and plant sources, has long been known to be an effective haemostatic agent and wound healing agent<sup>29</sup>. The potential use of this bio-inert material has been studied extensively and is now being modified to improve its wound healing properties<sup>269,270</sup>.

#### **2.1.12.2 Chitogel as a haemostatic agent:**

Valentineet al (2009)<sup>49</sup> conducted a study in 21 sheep infected with *Oestrusovus* that underwent standardised mucosal injuries in the sinuses. They evaluated the bleeding scores at Time = 0 (at time of mucosal injury) and every 2 minutes in Chitogel-applied mucosa vs. control (nothing) mucosa. It was noticed that Chitogel was a better haemostatic agent at 2, 4 and 6 minutes after injury. The average time to haemostasis was significantly better in Chitogel sides compared to control sides (4.09 vs 6.57 minutes,  $p=0.049$ ). Furthermore, complete haemostasis occurred within 6 minutes for all Chitogel sides, whereas control sides were still bleeding at 8 minutes in 3 sheep and 10 minutes in 1 sheep.

Valentine *et al* (2010)<sup>44</sup> followed on from the sheep study to demonstrate the same outcome in 40 human subjects undergoing ESS for CRS in which patients served as their own controls. Similarly, they scored baseline bleeding (Time = 0) immediately after the sinus operation, and every two minutes thereon. In that study, Chitogel achieved haemostasis within a mean time of 2 minutes (2-4 minutes) compared to 10 minutes for the control sides. Wound healing was also evaluated in the study and will be discussed in the section below.

### **2.1.12.3 Chitogel as a wound healing agent:**

Chitogel has been investigated in both animal and human models for its anti-adhesive properties. A prospective randomised controlled trial of Chitogel was performed in 20 sheep<sup>49</sup>. After creating standardised injuries and applying 3 types of haemostatic agents (Chitogel, SprayGel™ and recombinant tissue factor) compared to control in a randomised fashion, sheep were reviewed by a blinded observer monthly for four months. Results of that study showed that Chitogel was the only agent significantly superior to control in preventing adhesions. Chitogel was shown to reduce adhesions from 56% in the control group compared to 5% in the treatment group ( $p < 0.01$ ). Wound healing was determined by light and electron microscopy and measurements of ciliary beat frequency. The results of that study showed that Chitogel was significantly better (30% improved) than SprayGel™ group (13% improved), compared to control ( $p < 0.05$ ).

In the study mentioned above. Valentine *et al* (2010)<sup>44</sup> investigated the haemostatic effect of Chitogel in 40 humans undergoing surgery for adhesions and other factors of wound healing also post-operatively for up to 3 months post-ESS surgery. Following intra-operative bleeding evaluation, patients were followed up at 2, 6 weeks and 3 months where their adhesions, crusting, mucosal oedema, infection, and granulation tissue were graded. That

study indicated that there was a significant reduction in adhesions post-ESS compared to control. There was no significant difference in the other parameters.

Ha *et al* (2013)<sup>46</sup> in a prospective, randomised, controlled trial of Chitogel vs. control (nothing), in human patients, Chitogel on its own was significantly better in terms of reducing ostial stenosis post-ESS. The patients were evaluated at 2 weeks, 2 months and 3 months post-procedure by endoscopic measurements using a custom-designed ball probe. The results of that study showed that the frontal ostia maintained their diameters at 66% vs 31% for Chitogel vs controls. Similarly, the sphenoid ostia maintained their diameters at 85% vs 47%, and maxillary ostia at 74% vs 54% for Chitogel vs. control respectively (all  $p \leq 0.002$ ).

A follow-on study to these investigated the effect of Chitogel combined with Pulmicort Respule® (budesonide, 1 mg/2 mL) in the early post-operative period<sup>45</sup>. The budesonide solution was used for its anti-inflammatory properties in that study. Again, using patients as self-controls, Chitodex+budesonide gel was compared with control (nothing) and steroid-only (betamethasone cream). Preliminary results have shown that Chitogel with budesonide is superior to both control and steroid-only at improving healing in the early post-operative period. In the frontal sinuses, sides treated with Chitodex+budesonide gel maintained 71% of their ostial diameter at 12 months, compared to 51% in controls (no treatment). Similar results were seen in the sphenoid and maxillary sinuses, but less pronounced.<sup>78</sup>

#### **2.1.12.4 Chitogel as an anti-bacterial/anti-biofilm agent:**

Paramasivan *et al* (2014)<sup>80</sup> investigated the anti-bacterial and anti-biofilm effects of Chitogel in an *in vitro* study using fibroblasts isolated from human nasal tissue. They set out to determine the effects of Chitogel on (1) cell proliferation, (2) wound healing, (3)

inflammation in fibroblast cultures challenged with superantigen S, *S. aureus* enterotoxin B (SEB) and toxic shock syndrome toxin (TSST) and (4) *S. aureus* biofilms. They found that Chitogel was highly effective at reducing IL-8 expression after TSST and SEB challenge and non-challenged fibroblasts, indicating its anti-inflammatory effects on fibroblasts in the diseased state. Chitogel also showed strong anti-biofilm properties at 50% concentration and dextran on its own showed anti-biofilm properties at 1.25% concentration. Chitosan on its own reduced proliferation of fibroblasts to 82% of control and Chitogel reduced proliferation of fibroblasts to 0.04%. Furthermore, Chitogel significantly delayed wound healing rates over the first 2 days of wound-healing time.

In all these trials involving Chitogel as a topical product in human subjects, there have been no adverse effects.

### **3 Wound Healing in Abdominal Surgery**

#### **Introduction**

Abdominal surgery is performed for various indications both planned or in emergency situations via laparotomy over organ systems in the abdominal cavity. The more common are the surgeries done for the Gastro-intestinal(GI) system and Gynaecological surgeries involving the pelvic or the female reproductive organ system. Open laparotomy is deemed to be the primary cause of abdominal adhesions. An adhesion is a band of scar tissue that binds two parts of tissue together when they should remain separate. Adhesions may appear as thin sheets of tissue similar to plastic wrap or as thick fibrous bands. This tissue develops when the body's repair mechanisms respond to any tissue disturbance, such as surgery, infection, trauma, radiation. Tissue adhesions occur after 67% to 93% of abdominal surgeries,

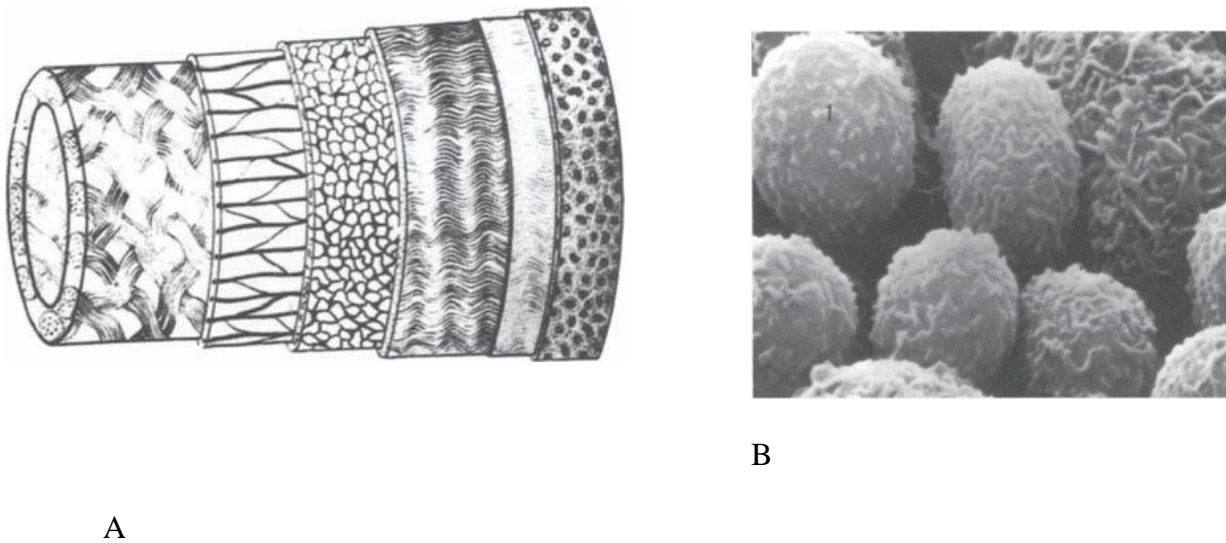


developing only hours after the operations<sup>271-273</sup>. The wound healing process is physiologically important; but adhesions, forming as part of that process are undesirable because they are associated with a reduced quality of life and can cause serious complications<sup>271,274</sup>. They form strangle holds over organs and cause chronic pelvic pain, infertility, urinary tract problems, sexual dysfunction, and bowel obstruction. Once the adhesions form, the risk of suffering from one or more of these associated problems is lifelong.

It has been reported that infections in the abdomen are one of the most potent stimuli for adhesion formation<sup>275</sup>, as seen clinically in patients with perforated bowel (such as severe appendicitis and diverticulitis) or contaminated operations (faeces leakage)<sup>276</sup>. Such infections often cause severe dense adhesions in the abdomen that result in bowel obstructions and need for subsequent operations to remove the adhesions, resulting in further morbidity and mortality<sup>277</sup>.

### **3.1.1 Anatomy & Physiology:**

The GI tract wall, except for the oesophagus and distal rectum, consists of 4 layers: mucosa, submucosa, muscularis propria, and serosa. The serosal layer is in contact with the peritoneum a membranous sac that contains the abdominal contents — parietal peritoneum and forms folds over the organs in cover – visceral peritoneum . Peritoneum is the most extensive serous membrane in the body and its area is generally equal to that of the skin and has 2 layers namely connective tissue and mesothelium<sup>278</sup>.



**Figure 11: A. Depicting the multiple layers of the Intestinal walls, B. Peritoneum – electron microscopy depicting mesothelial cells with microvilli, with permission(23)**

The main function of the peritoneum is to form a protective layer that help in the frictionless gliding of abdominal viscera and localize infection with the rich lymphoid organs in it and provide nutrition through the vasculature that passes through it. It also helps in storage of fat and contains numerous elastic fibres, especially in the deeper layer of the parietal peritoneum. The mesothelial cells that form the outer layer of the peritoneum are lined by microvilli in its outer surface that help is in the transport of the peritoneal fluid that is present in the abdominal cavity. The peritoneal fluid physiologically is present between 5 to 20 ml, it is rich in fibrinogen which increases in inflammatory conditions and also contains four types of differentiated cells: macrophages, mesothelial cells, Lymphocytes, and polymorphonuclear leukocytes. All these play an important role during injury and wound healing.

**Physiology of Wound healing in Abdomen:**

Mucosal injury of abdominal viscera during surgery is repaired by migration and proliferation of epithelial cells, thus sealing the defect and creating a barrier to luminal contents. Direct mucosal apposition is important for this process to occur quickly (as short as 3 days) as compared with mucosal eversion or inversion. Submucosa is the most important layer of the intestinal wall for surgeons because it provides most of the tensile strength and is the anchor for holding anastomotic sutures. The serosa being the outermost layer in the GI tract is made of connective tissue and mesothelial cells, blood vessels, and lymphatics. Wound healing on the serosal surface of injury is unique. Contrary to epithelial wound healing occurring from the wound edges, the injured site is covered by the formation of a sheath of mesothelial cells by day 3 closing the defect (by day 5)<sup>279</sup>. The other forms of peritoneal injury is by contamination of the peritoneal cavity at the time of operation with various materials such as glove powder, gauze fluff, suture material, and cellulose derived from disposable gowns and drapes. These substances can induce an inflammatory foreign-body reaction with consequent formation of granulomas and adhesions or both. Ellis et al reviewed the effect of glove powder, especially, because of it being blamed as a common cause<sup>279</sup>.

The term “adhesion” is used in the context of different types of injury. The formation of peritoneal adhesions is unique and specific to the peritoneal response to injury. Watters et al<sup>280</sup> in their seminal work in 1969, described the wound healing upon peritoneal injury by examining adhesions under SEM: on injury a fibrin matrix is rapidly formed within 30 minutes, and within 2 hours and over the course of the following 7 days fibroblasts and different immune cells infiltrate the fibrin plug, effectively forming fibrous bands (adhesions)<sup>281</sup>. At 7 days, fibroblasts and collagen form the main part of the adhesion as part of the proliferative phase and no further adhesions are formed. In the inflammatory stage of wound healing the role of the molecular interactions between the Polymorphonuclear cells

(PMN's), extra cellular matrix and the chemical signalling substances such as TNF and interleukins(IL) play a crucial role in the outcome of wound healing<sup>16</sup>. The role of IL-6 cannot be understated in this process<sup>282</sup>, it becomes a critical factor in switching the phase of wound healing process from being a prolonged inflammatory to the state of proliferation and then on leading to fibrosis or adhesion in the abdomen. The adhesions formed in the abdomen or pelvic region have a direct and proportional relation to the quantity of IL-6 production<sup>283</sup>. Hence, regulation or dysregulation becomes a critical factor in the outcomes of wound healing<sup>284</sup>.

Remodelling or Fibrinolysis in abdominal adhesion continues over several months, where the cellularity of the adhesion is gradually lost and replaced by collagen fibrils. Adhesion formation can be exacerbated by pathological processes such as infection and hematoma formation resulting in excessive fibroblast migration into the fibrin plug<sup>278</sup>. Optimal prevention of adhesion formation thus requires intervention during the critical 7-day period after surgery. Intra-abdominal contamination is inevitable in abdominal surgery, particularly in cases where the lumen of the bowel is penetrated. The main factor contributing to the subsequent development of intra-abdominal infection<sup>285</sup> is the quantity of bacterial contamination as a consequence of spill of colonic contents during the procedure and/or failure of the anastomosis occurring in 3–6% of all colon anastomoses<sup>286</sup>. Intra abdominal infection<sup>285</sup> is a serious clinical problem with a mortality rate between 5 and 50%<sup>287</sup> and is an important cause of secondary adhesion formation<sup>288,289</sup>. While surgical techniques and clinical care management have dramatically improved survival rates of IAIs from a mere 10% one century ago to on average 75% in the 21<sup>st</sup> century, the development of new potent antibiotics is unable to follow the rapidly increasing number of resistant bacteria<sup>287</sup>. An alarming 30-40% of microbes isolated in the context of IAI post-surgery, are resistant to

current antibiotic treatment regimens, severely compromising the therapeutic options available for these life-threatening complications<sup>290</sup>.

### **Post-Operative complications of Adhesion Formation**

Adhesion formation after surgery in the abdomen or the pelvis region is commonly seen in 50 % to 100% of patients who undergo surgery<sup>24,278</sup>. The type of surgeries that are performed include general surgical procedures, gynaecological, Urology or Oncology related. Adhesions are outcomes of wound healing after surgical trauma, either blunt or sharp, cold steel or heat related secondary to use of cautery/laser or post-operative complication such as faecal contamination because of dehiscence or secondary infection after an anastomotic leak<sup>291-293</sup>. Adhesions also form after pelvic inflammatory disease and are a very common clinical situation causing infertility, pain, and bowel obstructions<sup>294</sup>. This cannot be confirmed unless the patient develops complications for which they would undergo a surgery in case of intestinal obstruction or relook laparotomy as in conditions where relook laparoscopy is necessary to treat infertility<sup>24,59</sup>. Bowel obstruction caused by adhesions requires major abdominal surgeries to relieve and has significant impact on healthcare costs.

#### **3.1.2 Prevalence and consequences:**

Historically intra-abdominal adhesions were described in patients with tuberculosis on post-mortem<sup>295</sup>, subsequently they were found to be the causative factors in small bowel obstruction causing severe morbidity and mortality<sup>296</sup>. With modernisation and improvement in surgical techniques and surgical procedures, the rise of abdominal adhesions have risen from a mere 7% in 1932 to 60-70% by 1977<sup>293,297</sup>. The current advancements in surgery using minimally invasive techniques and key hole surgeries using laparoscopes has not reduced the burden<sup>298</sup>.

The presence of intra-abdominal adhesions may remain inconspicuous without any symptoms but becomes evident when they present with adhesive small bowel obstruction, volvulus or chronic abdominal pain. Surgical and Clinical Adhesions Research (SCAR) Group report by Ten Brooke et al <sup>24</sup> report approximately 9-15% incidence of small bowel obstruction by any cause in patients undergoing abdominal surgery in 92 studies and adhesion seemed to be the commonest cause of post-operative small bowel obstruction in 56% of those patients. In female patients, adhesions within the pelvis may result in infertility, ectopic gestation and chronic pelvic pain<sup>24,278,299</sup>.

Adhesiolysis and bowel resection(s), are the treatment of choice in patients presenting with symptoms related to adhesion which itself is riddled with inherent surgical and anaesthetic risk. LAPAD (LAParotomy or LAParoscopy and ADhesiolysis) <sup>300</sup> reported a high rate (62.9%) of adhesion releasing procedure being required for patients who were being treated for various abdominal surgeries of which 10.5% sustained an in-advertent bowel injury and this invariably increased the occurrence of sepsis and intra-abdominal complications, wound infections and longer hospital stay and expenditure related to care. Adhesiolysis has been reported to generate complications in 19% of patients with a mortality rate of 8% <sup>300</sup>; while secondary bowel resections expose patients to further risks of anastomotic leaks, short bowel syndrome and enterocutaneous fistulae<sup>24</sup>.

In Australia the National Emergency Laparotomy Audit <sup>198301</sup>, highlights that of 562 emergency laparotomies conducted between a 2 year period in 4 hospitals catering to 550,000 urban population, the most commonest was for Adhesiolysis (24.7%) and 10.5% of this high risk group died during or after care. The cost associated with adhesion- related readmissions following lower abdominal surgery in the UK is over £50 million/year (about \$AUS 100 million/year) while the cost for the US health care system is \$US 2.3 billion annually <sup>302</sup>.

With millions of abdominal surgeries occurring annually worldwide, a product that reduces adhesions will significantly benefit the cost and quality of health care globally.

**Challenges in treatment of Adhesion:** Despite advancements in detection and treatment, adhesions in the abdomen remain common in surgical practice. Perforation of bowel as a result of severe infections (e.g., appendicitis, diverticulitis) or inadvertent bowel injuries result in abdominal sepsis and higher likelihood and severity of adhesion formation, and the associated complications.

It has been estimated that the presence of pelvic or abdominal adhesions may prolong Subsequent abdomino-pelvic surgeries by an average of 21 minutes<sup>24</sup>, and has been reported to extend operating theatre time. Adhesions may also necessitate any subsequent procedures to convert from laparoscopic (minimally invasive) to open and have been associated with inadvertent enterotomy (surgical operation that creates a permanent opening through the abdominal wall into the intestine), resulting in higher complication rate associated with bowel perforation. Adhesive small bowel obstruction, inadvertent enterotomy at reoperation, prolonged operative times, increased clinical workload, and high financial costs are important adhesion-related problems that need to be addressed. These complications are serious and require substantial further treatment, thereby increasing both the trauma to the patient and the cost of the surgery. Current bio-surgical solutions for abdomino-pelvic adhesion formation are suboptimal and thus the need for a more effective product remains. Unfortunately, no currently available method of treating and preventing adhesions is completely successful, particularly for blocking the mechanism of adhesion formation (of which the most potent is infection).

## **Current options for adhesion prevention:**

Prevention of adhesion is directly dependent on injury over the peritoneum. This also depends on the surgical condition and various factors related around this. Numerous surgical techniques and treatment adjuncts have attempted to address the issue of adhesion formation, no agent has progressed to widespread clinical use due to lack of clinical efficacy or undesirable side effects.

### **3.1.3 Surgical techniques to prevent adhesion:**

Since laparotomy was associated with high occurrence of adhesion formation, it was thought that minimal access surgery would reduce the same. Unfortunately, the occurrence of adhesion has not changed but the intensity probably has. Diamond et al <sup>59</sup> found that 97% of patients who underwent second look surgery were diagnosed to have some form of adhesion. Even though it was not known if those adhesions were de-novo or were existing adhesions that underwent lysis. Nevertheless, it could be concluded that laparoscopy is less likely to cause adhesion de-novo than laparotomy.

Various surgical techniques have been in practice over the ages, gentle handling of tissues, avoiding fresh blood clots left intraperitoneally- hence normal saline / ringer lactate wash post-surgery<sup>303</sup>, keeping the serosa wet with NaCl<sup>304</sup>, minimising ischemia being the commonly followed steps.



### **3.1.4 Non-surgical Methods to prevent Adhesion**

Two principles have been extensively tested. Firstly, the local application of pharmacological agents that interfere with critical events in adhesion formation (18). These strategies have seen poor success rates mainly due to the rapid clearance from the abdominal cavity after local application<sup>293</sup>. Secondly, the use of barrier systems to keep injured abdominal contents separated during the healing process<sup>305</sup>. Shortcomings of these include reabsorption prior to completion of healing<sup>306</sup>, antigenicity<sup>307</sup>, anastomotic leakage<sup>307</sup> and wound dehiscence<sup>308</sup>. Devices made of non-absorbable materials may offer longevity but their advantages are offset by the potential induction/perpetuation of infection and the threat of migration<sup>307</sup>.

### **3.1.5 Research in abdominal adhesion prevention:**

\An ideal barrier agent should be a biocompatible substance that is sufficiently flexible to conform to the abdominal cavity and able to be used during laparotomy or laparoscopy. It should also be able to adhere to the peritoneal surface and remain in-situ for 5 to 7 days after the surgery. Moreover, it should prevent thrombin formation and hydrolyse, without leaving degraded residue that is pro-inflammatory in nature, persist during the critical re-mesothelialization phase, stay in place without sutures or staples, remain active in the presence of blood and be completely biodegradable.

There are currently many researched absorbable barriers in the form of solution or membranous substance. The Solution based barriers, are the following: Dextran (Hyskon®) solution (Pharmacia, Uppsala, Sweden), Oxidized regenerated cellulose (Interceed®, Ethicon, a Johnson and Johnson company, Sommerville, NJ ), Absorbable Adhesion Barrier; Ethicon,

and Adept (Shire GmbH and Co. KG) is a 4% icodextrin solution. Among the membranous variety are: Expanded polytetrafluoroethylene (Preclude®) - membrane, hyaluronic acid (HA) / carboxy methylcellulose (Septrafilm®) – membrane.

**Table5: Various anti-adhesion agents researched**

|                                                                                                                 |
|-----------------------------------------------------------------------------------------------------------------|
| <b>Fibrinolytic agents</b>                                                                                      |
| Thrombokinase, fibrinolysin, streptokinase, urokinase, hyaluronidase, chymotrypsin, trypsin, papain and pepsin. |
| Tissue plasminogen activators(t-PA) <sup>309</sup> and recombinant t-PA <sup>310</sup>                          |
| Thromboxane synthetase inhibitors: Imidazole and Ridogrel <sup>311</sup>                                        |
| Thrombin inhibitor (rec-Hirudin®), <sup>312</sup>                                                               |
| Anti-proliferative medications: Paclitaxel <sup>313</sup> and Camptothecin <sup>314</sup>                       |
| Polypeptides: lysozyme, polylysine, and polyglutamate <sup>315</sup>                                            |
| <b>Anti-coagulants</b>                                                                                          |
| Heparin, and Heparin with Amniotic membrane <sup>316,317</sup>                                                  |
| Low molecular weight heparin (Enoxaparin-Na),LMWH with Hyperbaric oxygen <sup>318</sup>                         |
| <b>Anti-inflammatory agents</b>                                                                                 |
| Low-dose aspirin <sup>319</sup>                                                                                 |
| Anti-inflammatory peptides: retinoic acid, quinacrine, or dipyridamole <sup>320</sup>                           |
| Antihistamines: Promethazine <sup>321</sup>                                                                     |
| Corticosteroids: dexamethasone, hydrocortisone and prednisolone <sup>322</sup>                                  |
| Non-steroidal anti-inflammatory drugs (NSAID): Ketorolac, Tolmetin, Ibuprofen and Indomethacin <sup>323</sup>   |
| <b>Antibiotics</b>                                                                                              |
| Systemic antibiotics (cephalosporins or tetracyclines) <sup>17</sup>                                            |

|                                                                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------|
| Peritoneal irrigation (cefazolin or tetracycline) <sup>324</sup>                                                                         |
| <b>Mechanical separation</b>                                                                                                             |
| <b>a) Peritoneal instillates</b>                                                                                                         |
| Crystalloid solutions: normal saline and Ringer's lactate <sup>278</sup>                                                                 |
| Viscous solutions: 32% Dextran-70 (Hyskon <sup>®</sup> ) Pharmacia, Uppsala, Sweden <sup>325</sup> – used through hysteroscopy           |
| Carboxymethylcellulose (CMC): high MW polysaccharide gel <sup>326</sup>                                                                  |
| Hyaluronic acid (HA): a naturally occurring glycosaminoglycan <sup>327</sup>                                                             |
| HA with phosphate-buffered-saline HAPBS: (Sepracoat <sup>®</sup> , Genzyme Corporation, Cambridge, MA) <sup>328</sup>                    |
| HA with iron 0.5% ferric hyaluronate gel: (Lubriccoat <sup>®</sup> , Lifecore Biomedical Inc. Chaska, MN) <sup>329</sup>                 |
| Auto-cross-linked hyaluronan solution or gel (ACP-gel) <sup>330</sup>                                                                    |
| N,O-carboxymethyl chitosan (NOCC): gel and solution <sup>59,331-333</sup>                                                                |
| <b>b) Barriers</b>                                                                                                                       |
| <b>i) Endogenous barriers:</b>                                                                                                           |
| (1) Fetal amniotic membranes <sup>333</sup>                                                                                              |
| (2) Peritoneal transplants <sup>334</sup>                                                                                                |
| (3) Omental grafts <sup>335</sup>                                                                                                        |
| <b>ii) Exogenous barriers</b>                                                                                                            |
| (1) HA with carboxymethylcellulose HA-CMC: (Seprafilm <sup>®</sup> , Baxter International Inc. <sup>336</sup>                            |
| (2) Adhibit <sup>™</sup> : gel used after cardiac surgery <sup>337</sup>                                                                 |
| (3) Adept <sup>®</sup> : is an intra-peritoneal fluid <sup>338</sup> – FDA approved in 2006 for general surgical and Gynaecological use. |
| (4) Polyethylene glycol-PEG: (SprayGel <sup>®</sup> , Genzyme Corporation, Cambridge, MA) <sup>339</sup> -                               |

|                                                                                                                          |
|--------------------------------------------------------------------------------------------------------------------------|
| (5) Poloxamer 407 (FlowGel <sup>®</sup> , poloxamer, Pluronic F-127; BASF Wyandotte Corp., Parsippany, NJ <sup>340</sup> |
| (6) Polytetrafluoroethylene: (Gore-Tex <sup>®</sup> ) <sup>341</sup>                                                     |
| (7) Fibrin glue: composed of fibrinogen, thrombin, calcium, and factor-VIII <sup>342</sup>                               |
| (8) Oxidized-regenerated cellulose-ORC: (Surgicel <sup>®</sup> ) <sup>343</sup>                                          |
| (9) Interceed <sup>®</sup> (TC7, Ethicon, a Johnson and Johnson company, Sommerville, NJ <sup>344</sup>                  |
| (10) Modified neutralized Interceed (nTC7), Ethicon, a Johnson and Johnson company, Sommerville, NJ <sup>345</sup>       |
| <b>iii) New agents</b>                                                                                                   |
| Films of polyethylene oxide and carboxymethylcellulose: (Oxiplex <sup>®</sup> , FzioMed, San Luis Obispo, CA)            |
| Pluronic F127/F68 alginate–bupropfen mixture (Sol–Gel <sup>®</sup> , Sigma-Aldrich, Saint Louis, MO, USA) <sup>346</sup> |
| Aloe vera gel <sup>346</sup>                                                                                             |
| <b>iv) Agents under research</b>                                                                                         |
| (1) Colchicine <sup>347</sup>                                                                                            |
| (2) Medroxyprogesterone acetate (MPA) <sup>348</sup>                                                                     |
| (3) Calcium channel blockers <sup>349</sup>                                                                              |
| (4) Phosphatidylcholine instillation <sup>350</sup>                                                                      |
| (5) Vitamin E <sup>351</sup>                                                                                             |
| (6) D-Penicillamine <sup>352</sup>                                                                                       |
| (7) Methylene blue <sup>353</sup>                                                                                        |
| (8) Pentoxifylline <sup>348</sup>                                                                                        |
| (9) Statin <sup>354</sup>                                                                                                |
| (10) Epidermal growth factor (EGF) <sup>355</sup>                                                                        |

Of all these agents that have been researched for the same purpose, the ones that have been recommended for use by FDA and have clinical relevance currently are: Interceed<sup>®</sup>, Adept<sup>®</sup>, Seprafilm<sup>®</sup> and Chitogel<sup>®</sup>,

#### **3.1.5.1      *Interceed<sup>®</sup>***

Oxidized regenerated cellulose (Interceed) is similar to its parent compound, Surgicel (Johnson & Johnson Medical, Arlington, TX, USA), this being absorbable material used regularly by surgeons to achieve and maintain haemostasis. Interceed is altered form of surgical and acts as a physical protective barrier and remains in the abdomen for a longer period of time compared to Surgicel by forming gelatinous protective layer covering the damaged peritoneum, limiting its involvement in adhesion formation during the first 10 days until natural re-epithelialization. The material can be easily applied at the time of laparoscopy and follows the contours of the organs without the necessity for sutures and disintegrates after a few days. Limitation of the product is need for adherence to strict protocol for its optimal efficacy, removal of all peritoneal fluid, adequate haemostasis and use of a sufficiently large piece with at least 3- to 5-mm margin around the raw area. All these being a difficult to strictly in every step, outcome is not consistent<sup>356</sup>.

#### **3.1.5.2      *Adept<sup>®</sup>***

Adept, Icodextrin 4%, developed by Baxter Healthcare Corporation (Deerfield, Illinois, 418 USA), is an approved product as barrier in Europe for use in both laparotomy and laparoscopy, and in the USA for gynaecologic laparoscopy with Adhesiolysis. It is a non-viscous, iso-osmotic and clear solution, which can be re-absorbed by the lymphatic system in

3-5 days and broken down by serum amylase. But the use has become less favourable because of the side effects and extravasation<sup>357</sup>.

### **3.1.5.3 Hyaluronic Acid (HA)**

HA is a polysaccharide and a major component of many body tissues and fluids. Aqueous solution of this polymer has a water-like or viscous consistency and is easily applicable in the abdominal cavity. It has been found to have increased viscosity by addition of ferric ion through chelation to FeHA.

Intergel (Lifecore Biomedical Inc., Chaska, MN) Adhesion Prevention Solution is FeHA, and is used as a single administration (300ml) after surgery in the peritoneal cavity. In both animal studies and Phase 1 & 2 Clinical trials, Intergel solution had a significantly lower number of de-novo adhesions and reformed adhesions than control patients at second-look laparoscopy ( $p < 0.001$ ). Intergel solution further decreased ( $p < 0.01$ ) the number of adhesions at the primary surgical site in comparison to Ringer lactate or no treatment. But, Tang et al<sup>358</sup> found a high rate of complications in the form of wound dehiscence in patients treated with Intergel.

### **3.1.5.4 Seprafilm®**

Seprafilm®, Baxter International Inc. is composed of hyaluronic acid with carboxymethylcellulose. It turns into a hydrophilic gel 24 h after placement and provides a protective coat for traumatized tissue for up to 7 days. Approved by FDA in 1996, it has been safe and effective similar to that of Interceed, but it has been found not to be effective because of handling issues<sup>359</sup>, dry Seprafilm is brittle and they stick to instruments<sup>360</sup>, and is not repositionable; and the wet film has poor mechanical integrity and cannot be manipulated over the bowel that has been cut open<sup>361</sup>.

Hence, there is a need for research for anti-adhesive agent that is easy to prepare and apply and has the flexibility to be used in both open and laparoscopic surgery.

### 3.1.5.5 Chitogel

Chitosan Dextran (CD) - Chitogel, a surgical hydrogel, has been tested in a variety of applications due to its inherent anti-haemostatic, anti-adhesive and wound healing properties<sup>44,49,80</sup>. Importantly, whilst it has been found to be effective in preventing the formation of adhesions after abdominal surgeries<sup>362</sup>, it has never been investigated in an infected abdomen model. There is an urgent unmet need for the development of new treatment regimens that will prevent the formation of adhesions and prevent the development of IAI, and New antimicrobial strategy/compounds are to be found.

#### *Research studies related to Chitosan:*

Chitogel has inherent haemostatic and anti-adhesive properties and an excellent safety profile, in animal and human studies in the sinuses<sup>44,252</sup>. Similarly, a murine and porcine model were studied using Chitosan as an anti-adhesive agent by Lauder et al<sup>332,362</sup>. In the murine model, eighty adults male Wistar albino rats were randomized to undergo a laparotomy as surgical procedure followed by caecal abrasion or anastomotic simulation by enterotomy of the cecum with primary closure no extra treatment or to receive Chitogel after the surgery and a third control group using varying dosages of Dextran alone solution. The rats were euthanased and examined at postoperative day 21 and adhesions were graded by an investigator blinded to the treatment groups, using a predetermined adhesion measurement score. They found a significant reduction of adhesion in the group treated with Chitosan in comparison to the untreated or control both in Laparotomy and abrasion alone, but they had

suffered multiple challenges in their experiments. 1. The adhesion created in the controls were not consistent, 2. The dextran was being dissolved in sterile water and the dosage of Chitosan was not consistent in all the animals treated with, 3. The total volume of gel applied in each treated rat was not consistent and 4. There were multiple deaths in the groups, especially in the control groups which makes the comparison inconsistent.

In the porcine model, twenty female domestic pigs were randomized to undergo surgery as laparotomy followed by ileocecal resection with ileo-colic anastomosis. Following which they left alone or received Chitogel at the time of surgery. At an initial postoperative 21 days, a laparoscopy was performed to grade the adhesions under anaesthesia following which Adhesiolysis was then performed and Chitogel applied to all animals. At day 42 animals were euthanized and adhesions graded using the previously validated scoring regimen. Researchers found the Chitogel treated pig's abdomen had reduced adhesions on laparoscopy. Subsequent, Adhesiolysis and application or re-application of Chitogel also decreased adhesion. This experiment was something that closely mimicked human surgical conditions and the comparison to resection and anastomosis provided an avenue to test the ability of the Chitosan to allow normal wound healing to occur and also reduce adhesions without causing any wound dehiscence or anastomotic failure. Minor inconsistencies were the dosage, volume and the viscosity of the gel applied. Even though there was enterotomy, there was no true infection that was created in the abdomen or there was no evaluation of the presence of infection that could be present in pathological conditions in human beings<sup>273,363,364</sup>.

### **3.1.6 Novel Anti – adhesion treatment:**

Deferiprone (Def) is an iron chelator approved for the treatment of thalassaemia major, a blood disease that is characterized by the release of high amounts of iron in the blood.



Gallium Protoporphyrin <sup>89</sup> is a non-iron analog of haem, the protein that is complexing iron in the blood and within cells. Our research has shown that GaPP has strong antibacterial effects<sup>96</sup>. In addition, it is also seen deferiprone also has strong wound healing effects <sup>85</sup>and enhances the antimicrobial effects of GaPP, also after these products are incorporated into the Chitogel<sup>365</sup>. Invitro studies showed that the anti-microbial effects of the Def-GaPP-Chitogel is as potent as antibiotic Ciprofloxacin (Cip) and Chitogel control against different microbial biofilms (MSSA, MRSA, MDR *P. aeruginosa* and *E. Coli* clinical isolates and reference strains)<sup>366</sup>. Chitogel along with Def-GaPP is safe and effective in vivo as seen in a large animal model sheep infected wound model of sinusitis<sup>97</sup>. Results showed (1) absence of any toxic effects, (2) highly significant reduction of *S. aureus* biofilms, (3) reduction in inflammatory cell counts and (4) improved wound healing in the Def-GaPP-Chitogel treated sheep compared to Chitogel treated control sheep<sup>97</sup>). This was also studied in a spinal surgery sheep model (non-infective model), Chitogel with Deferiprone alone in different dosages and untreated control. Post-operative recovery and clinical examinations of the sheep were uneventful for all sheep over a 3-month period following surgery. MRI and histopathology showed absent toxicity and significantly reduced adhesion scores of paraspinal muscle fibres to the dura at three months post operatively. No effects were observed in bone or dura healing in any of the sheep, indicating Def-Chitogel reduced adhesion formation without negatively affecting the healing process of the dura<sup>367</sup>.

### **3.1.7 Animal models for Abdominal Adhesion research:**

There are various models in literature that have been proposed to study the post-surgical intra-abdominal adhesion<sup>368</sup>, and are replete with differences. Hence, it is necessary to

identify an appropriate model that suits the pathology under study and the demonstrable outcome. The desired qualities in a model study the anti-adhesion properties is; 1. Consistent ability to create adhesion, 2. Reliability of the adhesion produced to be comparable with human pathology and 3. Reproducible by other investigators<sup>368</sup>.

The animal models researched thus far used three principles to create adhesion: injury, foreign materials, and ischemia. Injury models are the most widely used in research<sup>358,369</sup>. Injury models are the most widely routinely used in research, cecum and uterine horn are the primary targets because intestinal obstruction and infertility are the main complications that result due to adhesions. Causing a mechanical or chemical injury to either of the target site increases the chance of producing adhesion and thereby reliably replicating a human pathology to test. Multiple small and large animal models have been in use, among them mice or rat is preferred due to its ease of availability and handling with a lower cost<sup>368</sup>. Along with this choice of adhesion site & stimulus, their size if in a membranous form or volume in a solution form also play a major role because of the limited nature of available space in small animal models. Hence, for membranous or acidic agent's larger animal model like a rabbit or dog is preferred to elicit a reaction and response that is appropriate. In small animal models such as mice and rats, the abdominal wall is thin in comparison to other and also the volume for expansion is limited and will put the animal in distress if applied more than recommended. Choice of adhesiogenic stimulus is also important they are classified as primary and secondary; mechanical abrasion over the intestine or uterine horn using a standard gauze, spring loaded template or electrocautery are the preferred methods<sup>370</sup>. Among the secondary, chemical agents that initiate a foreign body reaction are used are, starch, talc, nitrogen mustard, formalin, phenol, Tetracycline and cefazolin<sup>371,372</sup>.

Haemostasis after surgery is a crucial step in wound healing and also adhesion formation, fibrin is the framework on which platelets adheres and thereafter formal maturation into an organised tissue and band. Amount of residual bleeding differs in surgical scenario and hence the model is required to have a similar situation, where as it is seen that in less than "meticulous," situations commercially available-Interceed barrier, does not have an optimal effect on adhesion<sup>373</sup>. Infection at the site of the wound is a real-world possibility during abdominal surgery after resection anastomosis or perforated appendix due to infection. And faecal material is a potent adhesiogenic and a challenge for anti-adhesive agent. Recreating that in an animal model is possible by either performing a enterotomy or resection anastomosis wherein a unknown quantity of faecal material could be split into the cavity to mimic the same, and very few studies have addressed this problem<sup>372,374</sup>.

Hence, there is need for designing a small animal model which recreates an abdominal adhesion with easily available substance such as kaolin, reliable and able to reproduce an infected abdominal wound. More challenging is to find an anti-adhesive agent which has haemostatic and antibiotic properties.

## 4 Wound healing in Spine Surgery

### Introduction:

Back pain either upper cervical or low back region is a bane of modern lifestyle is a leading disabling disease globally<sup>375</sup> in the 21<sup>st</sup> century. It is one of the top 10 most costly conditions to healthcare budgets, accounting for a combined \$34.6 billion in lost productivity and expenditures annually in the US<sup>376,377</sup>. Back pain results due to various causes, and they could be classified as arising from: Mechanical origin, Trauma/Fracture related, Inflammatory / Infective related, tumour or neoplastic caused or due to other systems. Those related to the mechanical causes are usually due to disc herniation or those that cause compression over the nerve rootlets causing radiculopathy or severe debilitating pain along the dermatomes of the nerve being compressed<sup>378</sup>.

Spinal surgery is often considered as a treatment where decompression of neural elements provides relief in most cases. However up to 40% of patients suffer significant post-operative ongoing pain known as failed back syndrome (FBS)<sup>379</sup>. In the USA, spinal fusion surgery was the highest aggregate hospital costs of any procedure (US\$12.8 billion) and in Australia from 2003 to 2013<sup>380</sup>, the fastest increasing surgical procedure for spinal stenosis was complex fusion, although the surgery provides no added benefit compared with decompression alone, it is more costly and associated with greater harms<sup>381</sup>. Based on Australian Bureau of Statistics data, FBS occurs in up to 6,660 of the 22,200 patients undergoing spinal surgery each year in Australia<sup>382</sup>. Epidural adhesions, formed after surgery, contribute to this pain through tension on neural elements in more than 80% of FBS patients with a direct relationship between the severity of adhesions and pain scores<sup>383</sup>. The accepted definition of FBS as defined by the International Association for the Study of Pain (IASP) is as follows:

*Lumbar (cervical) pain of unknown origin either persisting despite surgical intervention or appearing after surgical intervention for spinal (origin) pain originally in the same*

*topographical distribution*<sup>382</sup>.

There has been an increasing trend over recent years to offer surgery as a modality of treatment<sup>384</sup> and consequently we have seen a growing trend in FBSS after laminectomy<sup>385</sup>. Numerous strategies have been tested to reduce adhesion formation post spinal surgery, however, to date, no therapeutic approaches have been wholly successful and there are no Food and Drug Administration (FDA)-approved marketed devices available for this indication<sup>381,386,387</sup>.

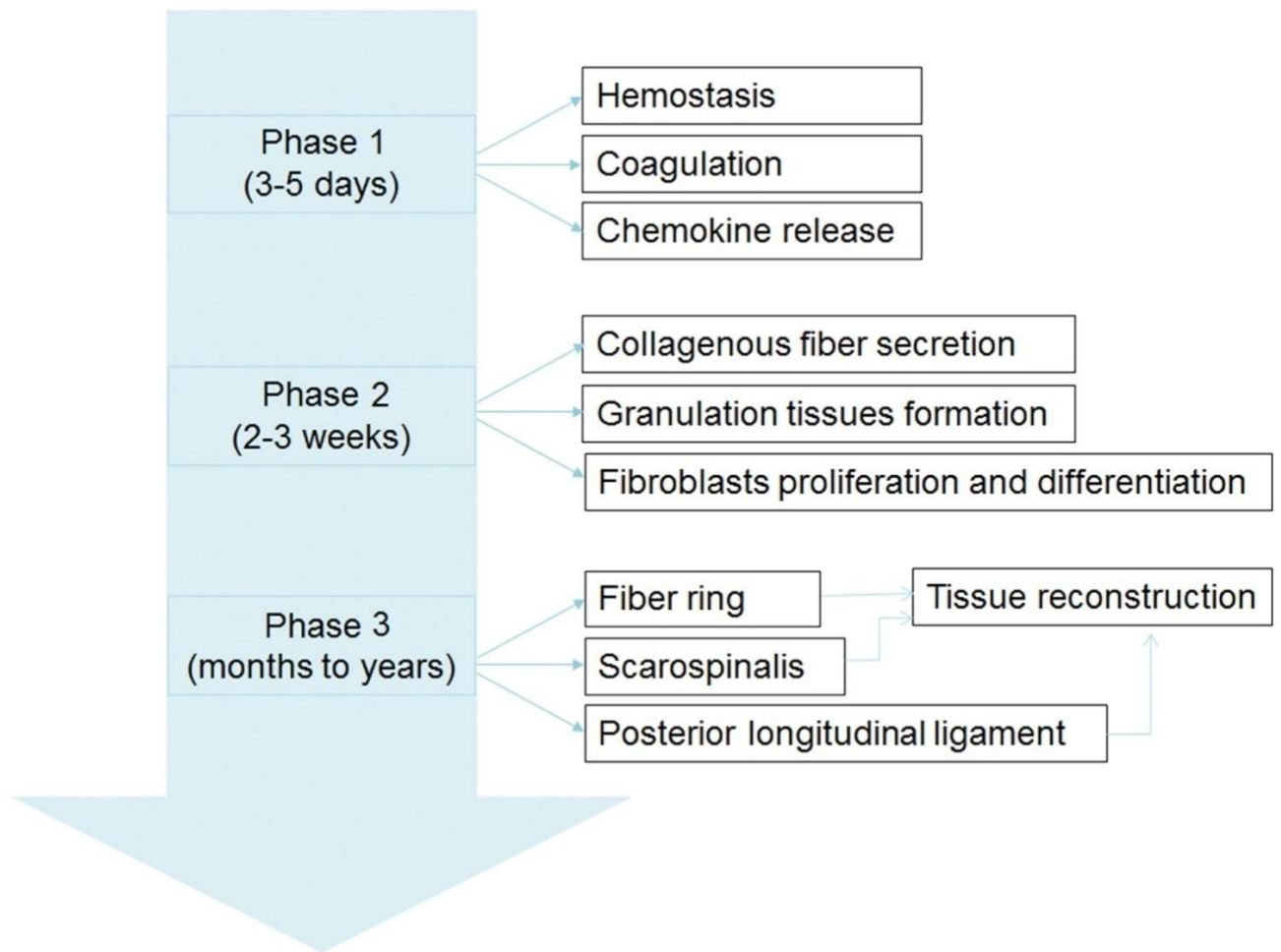
### **Patho-physiology of wound healing in the spinal canal:**

Understanding wound healing after spinal surgery is one of the crucial steps in treating the condition and to bring pain relief in FBSS. Epidural scar tissue formed in the surgical bed is unique in nature, wound repair by secondary intention is a multi-staged process which has been researched since 1948, Key and Ford<sup>388,389</sup> proposed the ‘annulus fibrosis theory’ where the diseased intervertebral disc was believed to be the source of fibrosis, much later LaRocca and Macnab in 1974 proposed the ‘Laminectomy membrane’ theory wherein he said the inner surface of the Sacro spinalis muscle could be the source of fibroblastic activity<sup>27</sup>. The current most accepted mechanism of fibrosis is as explained by Songor, Gosh & Spencer in 1990<sup>390</sup>. They explained it as a ‘three dimensional” process and the scar tissue around the dura mater originates from Sacro-spinalis behind, the fibrous ring and also posterior longitudinal ligament causing hyperplasia of fibrous tissue around the ventrolateral nerve root to cause epidural adhesion.

Normal wound healing is a highly regulated and coordinated process, in a narrow area such as the spinal canal however, tissue injury often results in scar tissue and adhesion formation and compression<sup>383</sup>. This adhesion formation can be exacerbated by pathological processes such as

infection, inflammation and haematomaformation<sup>391</sup>. Post-operative haematoma formation may result in excessive fibroblast migration into the clot<sup>391</sup>. It has been demonstrated that the critical time interval to block adhesion formation is primarily in the first 48 hours after the initial injury and the extent of adhesion formation is largely dependent on the inhibition of collagen production and of fibroblast proliferation and migration during that time<sup>25</sup>.

Haemostasis and coagulation are the first step in this process, bleeding from bone and muscles form clot, a major source of chemokine release such as phospholipase A2, which causes the aggregation of macrophagocytes, fibroblasts, mastocytes and endotheliocytes<sup>388</sup>. Fibroblast proliferation and formation of fibrocytes which secrete collagenous fibres in the defect and forms granulation tissue eventually is the second step which could take 2 to 3 weeks after initial insult. These fibroblastic activity in the extracellular space is regulated by various cytokines, such as transforming growth factor (TGF)-b1, interleukin-6 (IL-6) and fibroblast growth factor (FGF). Fibroblasts also secret TGF-b1, IL-6 and FGF-2 to improve fibroblast proliferation and extracellular matrix synthesis<sup>387</sup>. The third, tissue remodelling phase lasts months to years; fibrillar connective tissues deposit around the defect lesion and transform into scar tissues (Figure 12 ).



**Figure 12: Illustration of spinal canal woundhealingleading to adhesion formation<sup>387</sup>**

### Symptomatology, Diagnosis and Management:

The symptoms of lower back ache (LBA) and failed back surgery syndrome (FBSS) are due to entrapment of the nerve, either by the compression by herniated disc or in the abnormal scar that is formed after surgery. An accurate diagnosis is dependent on a thorough history, physical examination, and imaging of which MRI with Gadolinium with T1 is the Gold standard<sup>392</sup>. Many factors play a role into the formation of FBSS, they are psychological, bio-mechanical, surgical and a combination of these.

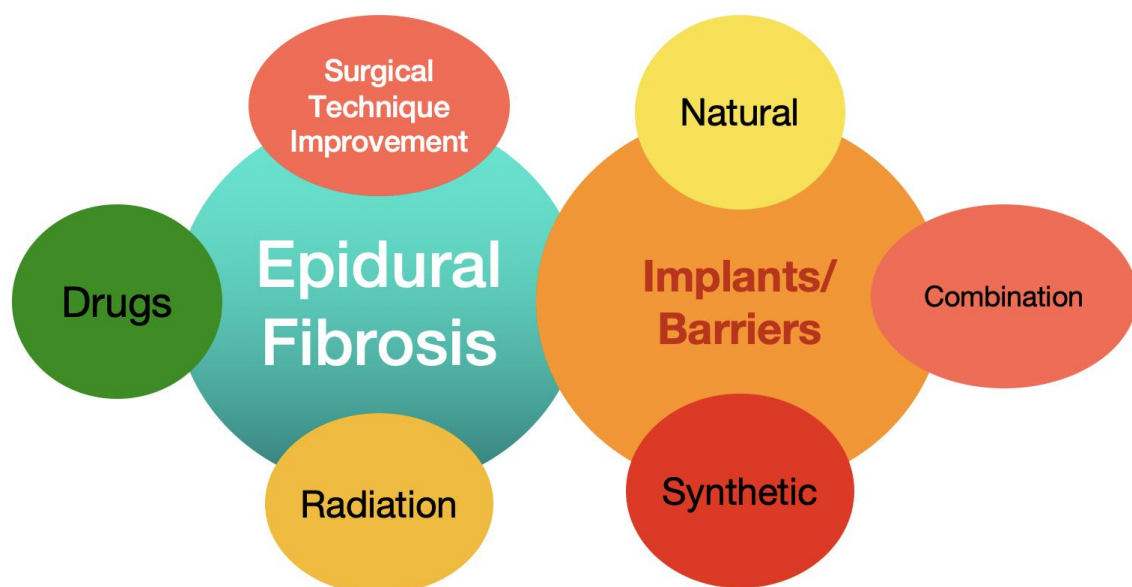
Treatment of FBSS includes a diagnostic nerve block and Physiotherapy as the first line of treatment<sup>393</sup>. Pharmacological therapy would be in the form of antiepileptics like Gabapentin

and Pregabalin for neuropathic pain, Non-steroidal anti-inflammatory drugs – Dexamethasone or methylprednisolone and opioids in preventing pain after surgery<sup>394,395</sup>.

When oral drug therapy does not resolve interventions that are painful like epidural injection of anti-inflammatory steroidal therapy is done. 3 commonly used routes are 1. Transforaminal, 2. Interlaminar, or 3. Caudal approach<sup>396</sup>, and use of this procedure has become increasingly common and abused as well<sup>397</sup>. Surgery becomes the only option when none of these interventions resolve the pain and misery that LBA causes to a person and his family.

### Current strategies to prevent epidural fibrosis:

The strategies used are modification of surgical technique, anti-inflammatory drugs and barriers placed between epidural space and muscular tissue.



**Figure 13: Illustration depicting the various treatment strategy to prevent spinal adhesion, adapted with permission 387**



### 4.1.1 Surgical methods:

Open Lumbar laminectomy is performed at any level with minor variations, during surgery after a posterior paraspinal incision the paraspinal multifidius and longissimus muscles are dissected off the spinous processes and held out to the width of the facet joints with the aid of retractors. Rongeurs and drill are used to remove the spinous processes and lamina. The ligamentum flavum is then exposed and removed, generally with a Kerrison punch. This exposes the thecal sac. From here the operation varies depending on pathology. On closing, haemostasis is achieved in the field and the paraspinal muscles re-approximated with sutures. The lumbar fascia is closed, and the extra fascial space obliterated with sutures. The skin and subcutaneous tissues are then closed. Patients are then recovered and, generally, ambulate as tolerated immediately.

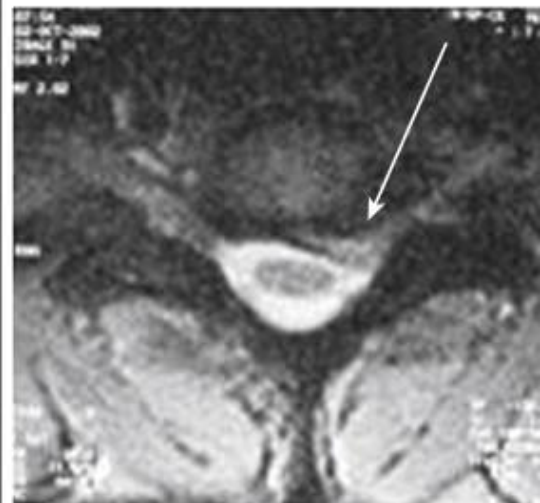
Minimally invasive techniques were devised to reduce the trauma and bleeding leading to subsequent spinal adhesion. Posterior cervical foraminotomy and micro-discectomy was one such procedure which is safe and effective this is used in the treatment of clinically significant foraminal stenosis resulting from lateral disc herniation or osteophytes<sup>398</sup>. Initially done through posterior approach was later changed to anteriorly due to its ease of use and many advantages, but now there is a radicle shift with advent of posterior micro-endoscopic foraminotomy (MEF)<sup>398</sup>. Where in the cervical pathology is visualised directly and the tissue destruction is minimal, muscle and ligamentous attachments to the spine are preserved<sup>184</sup>. Wu et al<sup>399</sup> in a systemic review and meta-analysis reviewed 26 studies including 2028 patients who underwent Full-EndoscopicPosterior Cervical Foraminotomy surgery (FE-PCF) and Micro-endoscopic Foraminotomy (MI-PCF) reported no significant difference in overall complication rate between the two. Dural tear is the most common undesired complication in

MI-PCF and transient neural palsy in FE-PCF. Hence, improvement of surgical technique alone does not prevent adhesion and FBS.

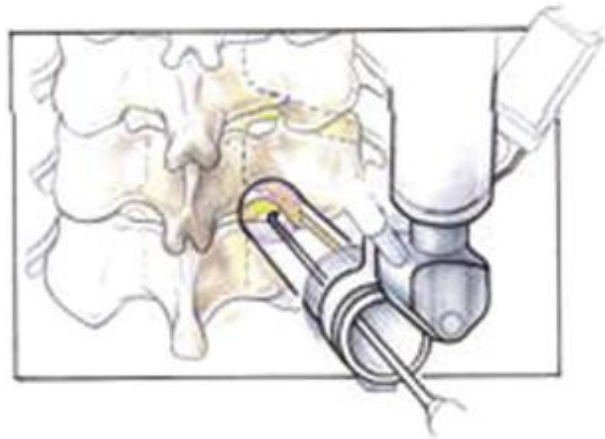


I

II



A



B



C

**Figure 14: Illustrative diagram showing the A. Cervical disc prolapse on an MRI, I - Sagittal view and II - Axial view, B. Location of pathology in relation to the lamina of the cervical vertebrae posteriorly, C. Placement of endoscope and excision of the compress**

### 4.1.2 Anti-inflammatory:

Prevention of adhesion formation in spine surgery is imperative since epidural scars can result in extradural compression, and tension on surgically exposed dura mater and nerve roots. This may cause recurrent radicular pain and physical impairment as soon as 6-12 weeks after surgery<sup>400</sup>. Because scarring is a necessary process of tissue repair, one possibility for avoiding post-laminectomy complications is to reduce the density and thickness of the scar tissue and limit adhesions to the dura mater and adjacent nerve roots without negatively affecting the process of normal wound healing. Administration of topical or systemic medications has thus far largely failed to prevent epidural scar tissue formation in animal models.

**Table 6: Various treatment options researched**

| Drug/Treatment           | Action                                           | Ref |
|--------------------------|--------------------------------------------------|-----|
| Mitomycin C              | Increases apoptosis of fibroblasts               | 401 |
| Dexamethasone            | Anti-inflammatory and reduces granulations       | 402 |
| Hydroxycamptothecine     | Anti-fibrocytes proliferation                    | 403 |
| Rosuvastatin             | Anti- fibrosis via inhibiting the TGF-1 $\beta$  | 404 |
| Radio frequency ablation | Ablation` of specific nerve rootlets             | 405 |
| Neuromodulation          | Spinal cord stimulation decreases pain sensation | 406 |

Topical steroids such as dexamethasone and budesonide are the main anti-inflammatory drugs that are used to target multitude of mechanisms faction that work synergistically and prevent leukocyte migration to areas of inflammation or injury. Steroids have also been used in combination with barrier methods, such as hydrogels. Chen et al<sup>407</sup> described an injectable hydrogel that was combined with dexamethasone with the aim of preventing epidural adhesions in rats. This study demonstrated a significant decrease in adhesions with the hydrogel barrier but that there was not a reliably significant decrease in adhesions with the addition of dexamethasone, nor was this dose dependant. This significant reduction in adhesions was only seen in the 2mg/ml dexamethasone concentration cohort. The authors attribute this to the possibility that the drug is not uniformly released from the hydrogel. The study would also seem to at least partially confirm the theory that, the migration of pro-inflammatory cells and direct contact of paraspinal tissue with the thecal sac results in adhesions and that a physical barrier to this may inhibit adhesion formation. It also provides some evidence for the use of anti-inflammatory medications in this barrier but there is conflicting evidence regarding dose and the issue of medication release from the barrier medium and requires further study. The probable explanation is the issue of mixing lipophilic steroid with a hydrophilic hydrogel and hence a delayed release.

#### **4.1.3 Barrier method:**

Barriers are the most commonly used method, and they are preferred due to the ease of use and better outcome; What makes an Ideal Barrier? An ideal barrier or scaffolding material should be one that could fill in the space created after surgery, bio-compatible and bio-degradable after a set time. Autologous fat has been used as a spacer clinically for long time with limited long-term benefit, hence natural polymers and synthetic polymers have been

tried<sup>408,409</sup>. Combination barriers are scaffolds incorporated with drugs that could give an added benefit. The polymers that have been studied as biomaterials in the last decade are cross-linked hyaluronic acid gel<sup>410</sup>, amniotic membrane<sup>411</sup> and silk-polyethylene glycol<sup>388,412</sup>. Most of these studies were designed to implant a synthetic or organic material into the laminectomy site as a barrier between the exposed dura mater and surrounding muscles - Silastic, Dacron, methacrylate, bone graft, synthetic membranes and foams, free and pedicle fat grafts, and steroid agents have been used<sup>387</sup>. There is, however, no consensus amongst spine surgeons as to the best and most effective option. Recently, surgical hydrogels have been developed and specifically marketed for the use to improve the clinical outcomes and prevent adhesions after spine surgery. With over 400,000 patients treated, FzioMed's surgical hydrogel for spine surgery, marketed under the brand names Oxiplex<sup>®</sup>, Oxiplex<sup>®</sup>/SP or MediShield<sup>™</sup>, is offered after spine surgery. It is proposed to serve as a protective physical barrier and is specifically marketed for reducing pain, lower extremity weakness, and the incidence, extent, and severity of postoperative adhesions after laminectomy<sup>377,413</sup>. Clinical trials have shown that coating the surgical site with Oxiplex improves clinical outcomes after spine surgery. However, due to efficacy data being discrete compared to control, only a confirmatory study approval has been granted to date despite FzioMed's repeated filings and appeals to the FDA for more than a decade<sup>377,414</sup>.

#### **4.1.3.1 Chitogel:**

Chitosan-Dextran (Chitogel) comprises succinyl-chitosan extracted from crustaceans and dextran-aldehyde has efficacious haemostatic ability<sup>415</sup> and anti-adhesive properties<sup>252</sup>. Chitogel may help to prevent scar tissue formation after laminectomy in human because of its haemostatic and wound healing properties and an excellent safety profile. Chitogel efficiently addresses haemostasis as well as adhesion prevention in sinus surgery; the first in its class to

do so. Chitogel has recently been FDA approved as a type III medical device in ENT surgery indications. Both in vitro studies and in vivo animal and human randomised trials have shown that Chitogel achieves haemostasis significantly quicker than control <sup>44,49</sup>, and reduces the incidence of post-surgical adhesions in endoscopic sinus surgery (ESS) <sup>44,252</sup>. Chitogel has also been tested in a burr hole neurosurgical sheep model and in a sheep laminectomy model confirming the excellent safety profile and haemostatic properties of Chitogel when applied to brain tissue <sup>416</sup> and its capacity to reduce adhesions after laminectomy <sup>417</sup>.

#### **4.1.3.2 Deferiprone (Def)**

Def is an iron-chelator, capable of chelating free iron at the ratio 3:1. Def has anti-microbial properties by capturing iron from the environment around bacteria, causing a depletion of iron as a nutrient source <sup>418</sup>. Mohammadpour et al have shown that Def can accelerate skin closure after topical Def application in vivo <sup>14</sup>. As specified by the FDA, the recommended dose for use for the treatment of Thalassemia Major is 75 mg/kg/day (up to 100 mg/kg/day). The long-term use of Def is associated with (reversible) agranulocytosis in 1–2% of patients and with less severe episodes of neutropenia in up to 5% of patients hence regular monitoring of neutrophil counts is recommended <sup>419</sup>.

The theoretical framework for the use of deferiprone to prevent adhesions stems from its ability to inhibit free-radical formation. Hydroxyl radicals are liberated by free iron in vivo <sup>470</sup>, <sup>471</sup>. These hydroxyl radicals are toxic to tissues. Experimental induction of free radical damage to hepatocytes by exposure to hydrogen peroxide has been shown to be reduced by concurrent exposure of deferiprone 1 mmol/L <sup>472</sup>. It has also been shown to prevent the oxidative damage from low density lipoprotein oxidation to blood vessels in rats and protect against reperfusion injury in rat hearts <sup>473</sup>, possibly through the inhibition of free-radical

formation, however human trials of these agents have no efficacy in the prevention or treatment of coronary heart disease<sup>474, 475</sup>.

Def has potent anti-inflammatory and anti-adhesive properties. Our in vitro assays have demonstrated that Def has potent dose-dependent effects on fibroblast migration and collagen production and efficiently blocks immune cytokine production in vitro.

#### Sheep model for experimental epidural fibrosis post-lumbar laminectomy:

The sheep model was chosen for our in vivo laminectomy studies because of our extensive practical knowledge of using sheep for preclinical studies, demonstrating suitability for in vivo spine surgery research<sup>417,420,421</sup> and because of the similar size and morphology of sheep and human spinal columns<sup>422</sup>. To induce adhesions, spinous processes and laminae are removed exposing the dura at different levels (Fig 5), followed by application of 2 ml 0.5g/ml Kaolin in normal saline<sup>423</sup>. Application of kaolin ensures extensive and consistent adhesion formation and can be assessed using macroscopic and histopathologic examination and imaging (MRI) (Jukes et al, manuscript in preparation).

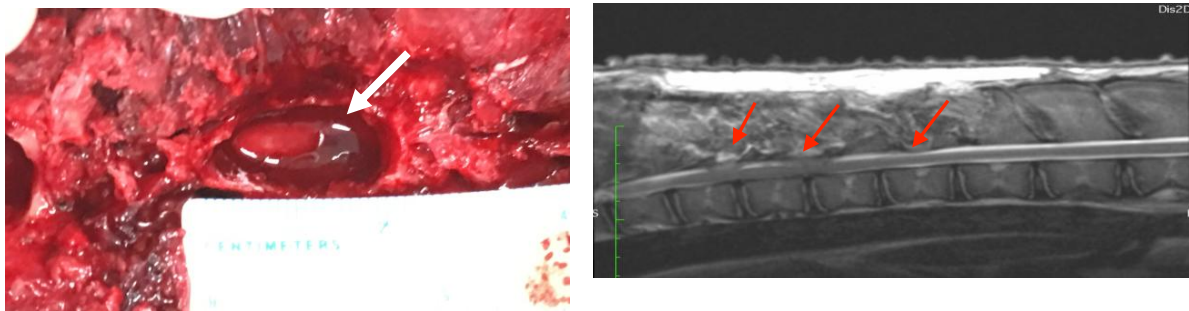


Figure 15: Illustration of laminectomy on sheep exposing the dura mater (White arrow) and MRI sagittal view after treatment (red arrow)



## **5 Summary of Literature review and research question:**

Surgical treatment of CRS, Abdomen and Spine have a common deterrent for a complication free outcome namely scar tissue or Adhesion. Currently there are no product or method available, which can effectively overcome the challenge along with delivering antimicrobial products in the site after surgery. This is proposed to be achieved by the use of Chitogel as a vehicle and scaffold for a novel anti-microbial combination of Deferiprone with Gallium Protoporphyrin. Furthermore, Chitogel has also been shown to have antibacterial and pro-wound healing properties in its own right, characteristics that may act in synergy in addition to the expected effects of Def and GaPP.

This knowledge and benefit in surgical outcomes could be transferred to other surgical specialities like abdominal surgery and spine surgery. Hence the research questions are.

### **Research Question:**

1. Does Chitogel with Deferiprone and Gallium Protoporphyrin have better post-operative outcomes in Chronic Sinusitis as compared to each of them alone?
2. Could post abdominal surgery adhesion be prevented with Chitogel with Deferiprone and Gallium Protoporphyrin?
3. Does Deferiprone with Chitogel have anti fibrotic effect on post-operative wound healing after laminectomy, if so at what dosage?

## 6 Chitogel with Def - GaPP in ENT-Endoscopic Sinus Surgery (ESS):

Summary: Rhinosinusitis (RS) the disorder of the upper airway and sinus cavities is characterised by the inflammation of the mucosa lining the nasal cavity and associated sinus cavities. Chronic Rhino-Sinusitis (CRS)<sup>424</sup> has multiple causative factors, many intrinsic in nature like anatomical defects, genetic and immune related abnormalities and much more extrinsic causes such as smoking and pollution that have been implied as cause<sup>425</sup>. Infection as an extrinsic causative factor has been proved beyond doubt and has been the main target of antibiotic treatment. CRS recalcitrant to treat despite multiple courses of antibiotic therapy and surgical management have been found to be due to Biofilms<sup>126</sup>. Biofilms as described by Costerton<sup>426</sup> are a niche of bacterial growth as a sessile community attached to substratum, and able to self-regulate growth and expression genetically. Foreman et al<sup>427</sup>, found a strong association between bacterial biofilms and sinusitis, *Staphylococcus aureus* was predominantly present in almost 61% of the RCRS patients<sup>247</sup>. These patients also presented with a higher degree of severity in symptoms and signs<sup>428</sup> on examination in comparison to those without biofilms.

Chitogel has been proposed to be used as a wound healing agent in the sinus cavity after an extensive animal study<sup>49</sup>, followed by human clinical trial<sup>44</sup> by Valentine et al. A follow-up study was designed and performed by Ha et al, where Chitogel was combined with a topical steroid solution (budesonide, Pulmicort®) or applied without steroid (Chitogel control) and applied in a similar manner following sinus surgery for similar patients<sup>45</sup>. Preliminary results of that study have shown reduced inflammation during the early postoperative period (compared to control) and even further improvements in ostial stenosis at 3 and 12 months. A previous study done using Mupirocin as a nasal irrigation flush was effective in the clearance of *S. aureus* infection in vivo and in patients with Saureus Chronic Rhinosinusitis<sup>248,429</sup>,

following this Mupirocin<sup>429,430</sup> and Budesonide in Chitogel gel showed good results in both antibiotic activity and also wound healing properties with the gel<sup>78</sup>.

Having studied Chitogel as a carrier for targeted delivery of antibiotics, the proposal is to use a novel drug combination discovered in our department to have synergistic antimicrobial properties (in-vitro & in-vivo)<sup>96</sup> Deferiprone, a compound that is TGA-approved for oral use to treat iron overload conditions such as Thalassemia, and Gallium Protoporphyrin, a haem mimetic. The combination of Deferiprone(Def) and gallium-protoporphyrin<sup>89</sup> has potent synergistic anti-microbial properties for different bacteria in planktonic, biofilm and small colony variant form including bacteria that are resistant to multiple types of antibiotics<sup>366</sup>. The combination Def-GaPP treatment is more efficient than first line antibiotic treatments to kill these bacteria. Gallium-protoporphyrin<sup>89</sup> is a compound that targets the bacterial nutrition by disturbing iron metabolism and eventually destroy it. Richter et al. reported on the in vitro activity of Def-GaPP against *Staphylococcus aureus* biofilms by interfering with bacterial iron metabolism. Def and GaPP can be incorporated into Chitogel and are efficiently released over a 2-week time period(in sheep studies)<sup>97</sup>. A second in vivo study was aimed to assess the safety and efficacy of Def-GaPP-Chitogel as a topical treatment against *S. aureus* biofilms in our sheep model of sinusitis. Incorporating this experience will allow to confirm the safety and efficacy of Chitogel in combination with Def and GAPP current standard of care post-operatively, which is oral antibiotics and nasal saline douching and determine applicability in preventing adhesions and reducing post-operative pain and disability in endonasal surgery.

### 6.1.1 Aim and Scope of Study

The specific aim of this study is

1. to investigate the safety and efficacy of Chitogel combined with Deferiprone and Gallium–Protoporphyrin, a novel formulation that acts on persistent infections and prevents the formation of adhesions.
2. to study the effect of Chitogel with Deferiprone as an anti-fibrotic agent.

The purpose of this study is to further develop a dissolvable dressing (Chitogel) that is designed to improve outcomes in patients with chronic rhinosinusitis (CRS). The ultimate goal is to develop a product that will encompass all aspects of postoperative care which also involves good wound healing and elimination of infection following operation for patients with CRS and other surgical specialties. This is proposed to be achieved by the use of Chitogel as a vehicle and scaffold for a novel antimicrobial combination Deferiprone with Gallium Protoporphyrin <sup>365</sup>. Furthermore, Chitogel has also been shown to have antibacterial and pro-wound healing properties in its own, characteristics that may act in synergism or addition to the expected effects of Def and GaPP.

### **6.1.2 Chitogel™ improves post-operative wound healing and patient outcomes in recalcitrant Sinusitis after ESS: Adding Deferiprone and Gallium Protoporphyrin, does it make a difference?**

Phase 1 Human Clinical Trial conducted in the Department of Otolaryngology – Head and Neck Surgery, The University of Adelaide, Adelaide, Australia

Financial assistance provided by The University of Adelaide, Commercial Accelerator Scheme.

And supported by The Hospital Research Foundation and The University of Adelaide under Australian Government Research Training Program Scholarship



**Human clinical trial evaluating Chitogel™ with adjuvants for post operative Wound healing in ESS**

|                               |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Journal:                      | <i>The Laryngoscope</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Manuscript ID                 | Iscope-20-3784                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Wiley - Manuscript type:      | Original Reports                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Date Submitted by the Author: | 17-Dec-2020                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Complete List of Authors:     | Vediappan, Rajan; Christian Medical College and Hospital Vellore, Department of ENT; The University of Adelaide Faculty of Health and Medical Sciences, Department of Otolaryngology, Head & Neck, Skull Base Surgery<br>Benette, Catherine; The University of Adelaide Faculty of Health and Medical Sciences, Department of Otolaryngology, Head & Neck, Skull Base Surgery<br>Cooksley, Clare; The University of Adelaide Faculty of Health and Medical Sciences, Department of Otolaryngology, Head & Neck, Skull Base Surgery<br>Bassiouni, Ahmed; The University of Adelaide Faculty of Health and Medical Sciences, Surgery- Otolaryngology, Head & Neck Surgery<br>Scott, John; Dalhousie University, Department of Otolaryngology- Head & Neck Surgery<br>Al Suliman, Yazeed; The University of Adelaide Faculty of Health and Medical Sciences, Department of Otolaryngology, Head & Neck, Skull Base Surgery<br>Lumyongsatien, Jate; Mahidol University Faculty of Medicine Siriraj Hospital, Department of Otorhinolaryngology Head and Neck Surgery<br>Morratti, Stephen; University of Otago, Department of Chemistry<br>Psaltis, Alkis; The University of Adelaide Faculty of Health and Medical Sciences, Department of Otolaryngology Head and Neck Surgery<br>Vreugde, Sarah; The University of Adelaide Faculty of Health Sciences, Surgery-ENT<br>Wormald, Peter-John; The University of Adelaide Adelaide Medical School, Department of Otolaryngology Head and Neck Surgery; Royal Adelaide Hospital, Skull Base Surgery |
| Keywords - Combo:             | Nose and paranasal sinuses < Head and Neck, Wound healing < Basic research < Facial Plastics/Reconstructive Surgery, Biomaterials < Laryngology                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |

SCHOLARONE™  
Manuscripts

John Wiley & Sons

## Statement of Authorship

|                     |                                                                                                                                                                                                                                                |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title of Paper      | Human clinical trial evaluating Chitogel™ with adjuvants for post-operative Wound healing in ESS                                                                                                                                               |
| Publication Status  | <input type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication<br><input checked="" type="checkbox"/> Submitted for Publication<br><input type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Submitted for Publication to The Laryngoscope                                                                                                                                                                                                  |

### Principal Author

|                                      |                                                                                                                                                                                                                                                                                                |      |            |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------|
| Name of Principal Author (Candidate) | Vediappan, Rajan Sundaresan                                                                                                                                                                                                                                                                    |      |            |
| Contribution to the Paper            | Conducted the Study, collected data, analysis, interpretation, drafting manuscript, editing and submission of manuscript.                                                                                                                                                                      |      |            |
| Overall percentage (%)               | 76%                                                                                                                                                                                                                                                                                            |      |            |
| Certification:                       | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |      |            |
| Signature                            |                                                                                                                                                                                                                                                                                                | Date | 17.12.2020 |

### Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above)
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

|                           |                                                            |      |            |
|---------------------------|------------------------------------------------------------|------|------------|
| Name of the Author        | Bennett, Catherine                                         |      |            |
| Contribution to the Paper | Conduct of study, Collection of data & drafting paper - 2% |      |            |
| Signature                 |                                                            | Date | 17.12.2020 |

|                                                                              |                                                                               |      |            |
|------------------------------------------------------------------------------|-------------------------------------------------------------------------------|------|------------|
| Name of the Author                                                           | Cooksley, Clare                                                               |      |            |
| Contribution to the Paper                                                    | Conduct of study, Collection of data & drafting paper - 2%                    |      |            |
| Signature                                                                    |                                                                               | Date | 17.12.2020 |
| Name of the Author                                                           | Bassiouni, Ahmed                                                              |      |            |
| Contribution to the Paper                                                    | Conduct of study, Collection of data, analysis of data & drafting paper - 1 % |      |            |
| Signature                                                                    |                                                                               | Date | 17.12.2020 |
| Name of the Author                                                           | Scott, John                                                                   |      |            |
| Contribution to the Paper                                                    | Conduct of study, Collection of data & drafting paper - 1 %                   |      |            |
| Signature                                                                    |                                                                               | Date | 17.12.2020 |
| Name of the Author                                                           | Al Suliman, Yazeed                                                            |      |            |
| Contribution to the Paper                                                    | Collection of data & interpretation - 1 %                                     |      |            |
| Signature<br>(by Principal Supervisor with accepted<br>format of authorship) |                                                                               | Date | 17.12.2020 |
| Name of the Author                                                           | Lumyongsatien, Jate                                                           |      |            |
| Contribution to the Paper                                                    | Collection of data & interpretation - 1 %                                     |      |            |
| Signature<br>(by Principal Supervisor with accepted<br>format of authorship) |                                                                               | Date | 17.12.2020 |
| Name of the Author                                                           | Moratti, Stephen                                                              |      |            |
| Contribution to the Paper                                                    | Conceptualization, conduct & important intellectual content - 1 %             |      |            |



|                           |                                                                                                                                                                  |      |            |
|---------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------|
| Signature                 |                                                                                                                                                                  | Date | 17.12.2020 |
| Name of the Author        | Psaltis, Alkis                                                                                                                                                   |      |            |
| Contribution to the Paper | Design, Conduct, revising it critically for important intellectual content and approval for submission - 5 %                                                     |      |            |
| Signature                 |                                                                                                                                                                  | Date | 17.12.2020 |
| Name of the Author        | Vreugde, Sarah                                                                                                                                                   |      |            |
| Contribution to the Paper | Design, Interpretation of results, revising it critically for important intellectual content and approval for submission - 5 %                                   |      |            |
| Signature                 |                                                                                                                                                                  | Date | 17.12.2020 |
| Name of the Author        | Wormald, Peter-John                                                                                                                                              |      |            |
| Contribution to the Paper | Conceptualization, Design, Conduct, Interpretation of results, revising it critically for important intellectual content and final approval for submission - 5 % |      |            |
| Signature                 |                                                                                                                                                                  | Date | 17.12.2020 |

## **Human clinical trial evaluating Chitogel™ with adjuvants for post-operative Wound healing in ESS**

Rajan Sundaresan Vediappan MBBS, DLO, MS(ENT)<sup>1</sup>, Catherine Bennett BMedSc<sup>1</sup>, Clare Cooksley PhD<sup>1</sup>, Ahmed Bassiouni MBBS, PhD<sup>1</sup>, John R Scott MD<sup>2</sup>, Yazeed A. AL Suliman MBBS, SB-ORL<sup>1</sup>, Jate Lumyongsatien MD<sup>3</sup>, Stephen Moratti PhD<sup>4</sup>, Alkis J Psaltis PhD, FRACS(ORL)<sup>1&</sup>, Sarah Vreugde MD, PhD<sup>1&</sup>, Peter-John Wormald MD, FAHMS, FRACS, FCS(SA), FRCS (Ed), MBChB<sup>1&</sup>

<sup>1</sup>Department of Surgery - Otolaryngology Head and Neck Surgery, The University of Adelaide

<sup>&</sup>Department of Otolaryngology Head and Neck Surgery, Central Adelaide Health Network.

<sup>2</sup>Department of Otolaryngology- Head & Neck Surgery, Dalhousie University ,  
Halifax, Nova Scotia, Canada

<sup>3</sup> Department of Otorhinolaryngology Head and Neck Surgery, Faculty of  
Medicine Siriraj hospital, Mahidol university, Bangkok 10700, Thailand

<sup>4</sup>Department of Chemistry, Otago University, Dunedin, New Zealand

### **Corresponding Author:**

Professor Peter John Wormald

The University of Adelaide

Department of Surgery - Otolaryngology, Head & Neck Surgery

The Queen Elizabeth Hospital

28, Woodville Road, Woodville south-5011

Adelaide, South Australia

Contact: peterj.wormald@adelaide.edu.au,

Ph: (+61 8) 8222 7158 Fax: (+61 8) 8222 7419

**Author Contributions:** RSV, CB, CC, AB, JS, JL, & YS were involved in the conduct of the study, collection of data and interpretation of the results and with drafting and editing the manuscript. PJW, SV, SM & AP were involved in designing the study, supervision, interpretation of data, drafting and editing the manuscript and final permission for submission.

**Conflict of Interest:** PJW and SV are inventors on intellectual property concerning Gallium Protoporphyrin and Deferiprone for use in the prevention of scarring; PJW and SM are shareholders in Chitogel

**Funding:** This project is supported by The University of Adelaide Commercial Accelerator Scheme.

**Acknowledgements:** RSV was supported by The Hospital Research Foundation and The University of Adelaide under Australian Government Research Training Program Scholarship; the authors would also like to appreciate the contribution of The Queen Elizabeth Hospital, The Memorial Hospital for their Basil Hetzel Institute, Adelaide and their support during the study.

**Abstract:**

**Background:** Ostial stenosis and persistent inflammation are the main reasons for revision endoscopic sinus surgery (ESS). Post-operative (PO) dressings can improve PO wound healing and patient outcomes after ESS. This study aimed to determine the safety and efficacy of Chitogel, with and without Deferiprone (Def) and Gallium Protoporphyrin <sup>89</sup>, as a promoter of wound healing to improve surgical outcomes.

**Methods:** A double-blinded, randomized control human clinical trial was conducted in patients undergoing ESS as treatment for CRS. Participants were randomised to receive test product Chitogel, Chitogel in combination with Def or Def-GaPP versus no packing (control). Patients were followed up at 2-, 6- and 12-weeks PO, outcome scores such as SNOT-22, VAS and LKS, pre- and post-surgery (12 weeks) were compared.

**Results:** 79 patients completed the study, there was a significant reduction in SNOT-22 score and improvement of VAS at 12-week in patients treated with Chitogel compared to control ( $p < 0.05$ ). In those patients, the mean ostium area for the Chitogel and the Chitogel + Def + GaPP groups were higher across all 3 sinuses compared to the no-treatment control group, without statistical significance. Sphenoid sinus ostium was significantly more patent in patients treated with Chitogel compared to control at the 12-week time point ( $p < 0.05$ ).

**Conclusion:** Chitogel is an excellent post-operative dressing after ESS and results in the best patient reported symptom scores and objective measurements. The combination of Def and GaPP to Chitogel though proving safe, had no effect on the ostium patency or mucosal healing.

**Clinical Trials Repository :** 2018-01-28, CT-2017-CTN-04279-1-v1 Trial Chitosan-dextran (Chitodex) gel with and without Deferiprone and Gallium-Protoporphyrin: wound healing (University of Adelaide (Department of Otorhinolaryngology / Head and Neck Surgery))

**Keywords:** chronic rhinosinusitis; Chitogel™; absorbable packing; biomaterials; drug-eluting stents; endoscopic sinus surgery; haemostasis; nasal packing; removable packing; rhinology; wound healing.

Level of Evidence: Level 2

### **Introduction:**

Endoscopic sinus surgery (ESS) is the standard of care for chronic rhinosinusitis (CRS) patients who do not respond adequately to appropriate medical therapy<sup>431-433</sup>. The guiding principles of ESS are to improve aeration of the sinuses and to enable delivery of topical therapy to the sinuses<sup>200,434,435</sup>. Surgical outcome depends not only on adequate surgical clearance of obstructing cells and disease, but also on the post-operative delivery of medication to promote mucosal wound healing<sup>251,252</sup>. Success of ESS is determined by patency of the sinus ostia, health of nasal mucosa and the absence of repeated infections<sup>253,436</sup>. Failure of ESS is usually marked by ostial stenosis, adhesions, repeated infections and excessive granulation formation<sup>214,437</sup>. Adhesions occur in 10 – 30 % of patients undergoing ESS<sup>214</sup>; scarring and narrowing of frontal sinus ostia is seen in 60% of these patients leading to abnormal drainage patterns and persistent infections making this recurrent sinusitis difficult to treat<sup>215</sup>.

Traditionally, removable nasal packings were used to improve wound healing and control bleeding after surgery, but in recent years they have been replaced by absorbable nasal packings<sup>29,218,438,439</sup>. Some of these also provide drug delivery to promote wound healing<sup>439,440</sup>. Of all the sinus ostia, the frontal sinus remains the most challenging to keep patent and healthy post-operatively. Currently standard middle meatal stents do not reach the

mucosa of the frontal ostium or frontal recess but are placed between the middle turbinate and the lateral nasal wall.

Previous studies using Chitogel with and without steroid have shown significant benefit in both the preservation of the sinus ostia and improved wound healing<sup>45</sup>. Deferiprone (Def) is an FDA-approved iron chelator for use in the treatment of iron overload in thalassemia patients<sup>81</sup>. It also has anti-bacterial activity, by chelating the iron in the bacteria's environment which is required for metabolism and replication<sup>365</sup>. Another feature of Def is that it inhibits fibroblast migration, potentially providing anti-adhesion properties<sup>85</sup>. Gallium protoporphyrin<sup>89</sup> is a haem analogue. Haem is the primary source of iron for bacteria in the human environment. The structure of haem and GaPP is identical except that for GaPP the central ion is gallium, rather than iron. The bacteria recognise GaPP as haem, absorb it but are unable to metabolize it. The result is a release of reactive oxygen species and starvation leading to bacterial death<sup>96</sup>.

The aim of this study was to evaluate whether Chitogel alone or incorporating Def, with and without GaPP, was safe and could improve sinus ostium patency and mucosal healing in patients after ESS.

### **Materials and Methods:**

This prospective, double blinded, randomised controlled trial was approved by the Human Research Ethics Committee of the Central Adelaide Local Health Network (32707-HREC/17/TQEH/245; CALHN: Q20171012) and was conducted from February 2018 to December 2019. Patients over 18 years of age undergoing ESS with a diagnosis of CRS<sup>99</sup> and not allergic to shellfish/drug, pregnant or breastfeeding, without history of hepatitis or blood disorders were recruited in the study after informed consent was obtained.

Study Design: All participants underwent bilateral ESS with meticulous mucosal preserving technique using cold steel and powered instruments. Patients were separated into 2 main groups based on whether or not a frontal drillout (DO) procedure was planned in addition to the ESS. Patients in the full house FESS group (hereafter referred to as the FHF) were randomised to receive treatment on one randomised side of the sinuses in the form of Chitogel, Chitogel with Def (20 mM) and GaPP (250 µg/ml), Chitogel with Def (20 mM) or Chitogel with GaPP (250 µg/ml) and the opposite side was considered as control receiving no nasal packing as per Figure 1. Patients in the full house FESS +DO group (here after referred to as the drillout (DO) cohort) were stratified by block randomization to ensure equal numbers of patients in each of the treatment groups. Randomisation occurred in 4 groups: no packing control group, Chitogel, Chitogel with Def (20 mM) and GaPP (250 µg/ml) and Chitogel with Def (20 mM) (Figure 1).

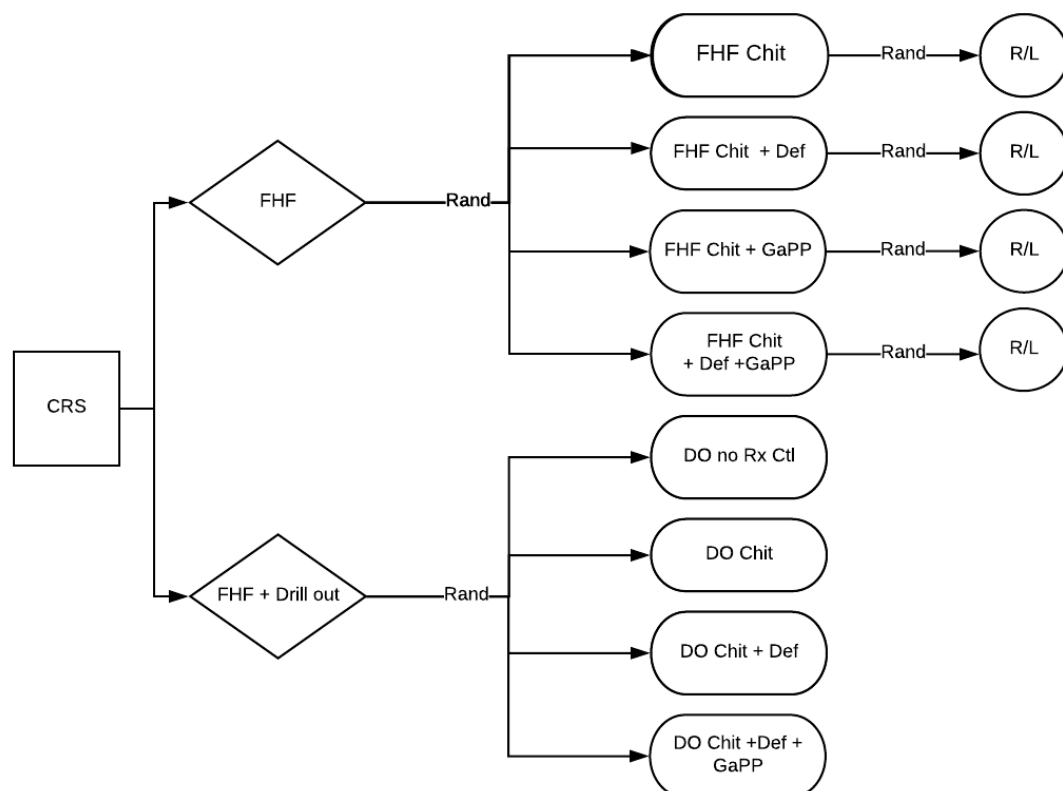


Figure 1: Schema of participant randomization (Rand) into FHF and FHF+DO groups (DO) for various treatments (10 patients in each group). FHF-Full House FESS; DO = drill-out +FHF; Chit = Chitogel; Def=Deferiprone; GaPP= Gallium Protoporphyrin; no Rx Ctl= no treatment control; R/L= right/left side.

Statistical power calculations: Power calculations were based on the requirement that effects be assessed at the 5% alpha level with 80% statistical power. The outcomes were measured based on the wound healing in the sinus openings made during surgery. We determined that a clinically meaningful difference between the two closest treatment groups would be a minimal difference of 3 mm<sup>2</sup> area<sup>441</sup> in the frontal ostial size at 12 weeks post-surgery. Based on available data, we expected the standard deviation to be approximately ½ the magnitude of the mean difference (i.e.: 1.5 mm). According to these values, 10 patients in each of the groups are needed to reach statistical significance. In this study, observations over time were correlated due to clustering and randomised by GraphPad Quickcalcs software (<http://www.graphpad.com/quickcalcs/index.cfm>).

### Details of the Active Compounds:

**Chitogel:** Chitosan and dextran aldehyde components were supplied by Chitogel Pty Ltd (Dunedin, New Zealand) in sealed containers after sterilized during the production process.

**Deferiprone:** (3-hydroxy-1,2-dimethylpyridin-4(1*H*)-one) was sourced from Sigma-Aldrich (St Louis, MO, USA) and used at a final concentration of 20mM in Chitogel.

**Gallium Protoporphyrin IX** (Ga-PP IX) was sourced from Frontier Scientific (Logan, UT, USA) and used at a final concentration of 250 µg/ml in Chitogel.

Def and GaPP were dissolved in 0.3% sodium hydrogen phosphate buffer solutions and 40% glycerol in sterile conditions. Once dissolved, the solution was filter sterilised in an aseptic



environment using 0.22 micro syringe filters. Sterile Def and GaPP stock solutions were stored at room temperature, protected from light and used within 4 weeks.

***Application of treatment and post-operative care:*** At the end of the FHF procedure, 20 ml of gel (Chitogel or Chitogel + Def or Chitogel + GaPP or Chitogel +Def + GaPP) was instilled using the supplied malleable applicator into one side of the patient's sinuses selected by computer randomization. In the FHF plus drill out (DO) procedure group, patients received no treatment (control group) or 40ml Chitogel, Chitogel + Def or Chitogel + Def + GaPP. Post-operative care proceeded as per standard protocol for ESS surgery.

***Blood tests:*** Drug safety profile was evaluated by adverse event reporting and by testing total blood cell counts, liver function tests and serum ferritin levels pre-surgery and 2 weeks after surgery.

**Follow up protocol and outcome measurements:**

Post-operatively, all participants received an empirical course of antibiotics as this is our routine standard of care. Evaluation of both nasal cavities was performed at 2, 6 and 12 weeks after surgery. At review, cleaning of the sinuses and a recording of their endoscopic sinus examination was carried out and participants were instructed to perform steroid-saline douches daily on each side.

***Subjective and objective outcome measurements:*** Pre and post-operative questionnaire-based evaluation was done at 0 and 12 weeks post-operative. This included subjective symptom scores using the SNOT-22 score and Visual Analog Scale (VAS) patient reported scores (0-10) for evaluating the facial pain/discomfort, bleeding, nasal obstruction and nasal secretions on both the left and the right side. At each visit, all participants underwent nasal endoscopy which was recorded, and these endoscopic videos were graded by blinded independent

surgeons using the Modified Lund Kennedy Score (LKS)<sup>136</sup>. A consensus ordinal scale score was given for crusting, mucosal oedema, infection, granulations and adhesion. During the post-operative visits, on evidence of persistent infection, a culture-directed course of oral antibiotic was given.

***Measurement of ostial openings:*** Evaluation of ostial dimensions was performed by taking measurements from the 3 binary pairs of maxillaries, sphenoid and frontal sinus ostia at the end of the surgery just before the application of the gel. The measurements were repeated in clinic, on all sinus ostia at 12 weeks post-surgery, using a previously standardized technique<sup>46,78</sup>.

### **Statistical Methods:**

The results of all variables were collected and compared between treatment groups at 2 time points: 0 weeks-intraoperative baseline and 12 weeks post-surgery. These were collated to produce a mean value with standard deviation for each post-operative time point. Data for SNOT-22, VAS and LKS were analysed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria) through the Jupiter notebook interface. The R package "MASS"<sup>442</sup> was used for ordinal regression. Statistical significance was taken at the traditional  $< 0.05$  level. Linear mixed model was used for t-tests Satterthwaite's method (longitudinal ANCOVA) and results are averaged over the levels of time factor and Degrees-of-freedom method Kenward-roger using a Confidence level used: 0.95. P value adjustment was done by the Tukey method for comparing a family of 20 estimates was used with "treatment" defined as Full House FESS group were FHF Control - FHF no Rx Ctl, FHF Chitogel alone - FHF Chit, FHF Chitogel plus Def – FHF Chit +Def, FHF Chitogel plus GaPP - FHF Chit +GaPP, FHF Chitogel plus Def and GAPP - FHF Chit +Def + GAPP and similarly FHF + Drillout Control - DO no Rx Ctl, FHF + Drillout with Chitogel alone - DO

Chit, FHF + Drillout with Chitogel plus Def - DO Chit +Def and FHF + Drillout with Chitogel plus Def and GaPP - DO Chit + Def +GaPP (Table 1 and 2). Data for ostial measurements were collated in mm<sup>2</sup> for original intra-operative measurements and as percentages at weeks-“0” intra-operative and 12 weeks Post-operative and analysed using Kruskal Wallis for DO and Wilcoxon for FHF group. Graphical representations are displayed in Figures 2-6.

### **Results:**

From a total of 82 patients recruited into this trial, a total of 79 patients (47 male, 32 females, aged 18-80 years) completed the trial, of which 40 underwent FHF and 39 FHF with DO, 3 patients failed to complete the study. Details of patient demographics are shown in Supplementary Table 1. The 40 patients in the FHF group were randomized to receive treatment on one side with the other side acting as a within patient control. In the FHF+DO group, 39 patients were randomised to receive no treatment (control) or treatment – Chitogel only, Chitogel with Def and Chitogel with Def and GaPP. There were no reported adverse events or complications during the trial.

### **Comparison of patient symptom scores and SNOT 22 in Full House FESS (FHF) + Frontal Drill Out (DO) group**

Patients who underwent FHF with DO treated with Chitogel alone had a significant improvement in SNOT 22 scores compared to control patients at 12 weeks after surgery (p=0.048). A similar trend was observed in the Chitogel with Def group as compared to control, but the results were not statistically significant (p=0.13). Patients treated with Chitogel with Def and GaPP did not have any significant differences compared to control in SNOT 22 scores (p=0.39). Results are detailed in Table 1A and Fig 2.

**Table 1A. Patient SNOT-22 scores (mean and SEM) in each treatment group after FHF with Drill Out (FHF with DO)**

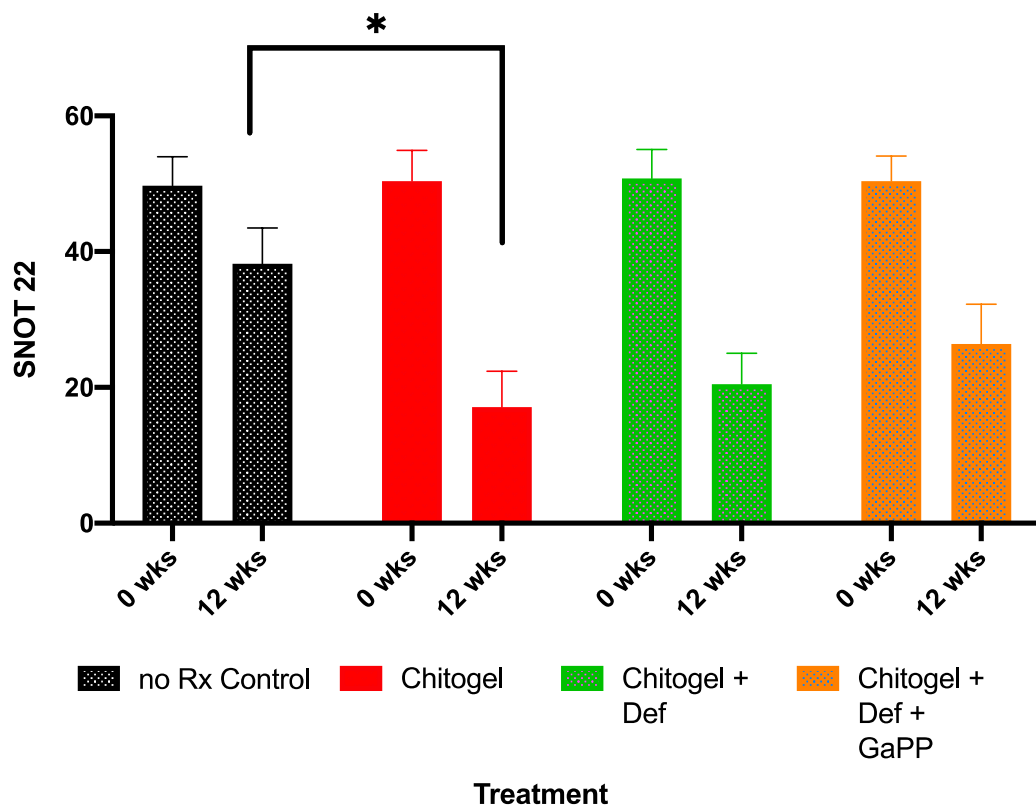
| FHF with DO, treatments received | Mean SNOT-22 Score Pre-Op - 0 week | Mean SNOT-22 Score Post-Op 12 week | p-value of linear Mixed Model, longitudinal ANCOVA control Vs treatment |
|----------------------------------|------------------------------------|------------------------------------|-------------------------------------------------------------------------|
| no Rx Ctl                        | 49.7 ± 4.25                        | 38.2 ± 5.30                        | -                                                                       |
| Chit                             | 50.4 ± 4.51                        | 17.1 ± 5.28                        | 0.048                                                                   |
| Chit +Def                        | 50.8 ± 4.25                        | 20.5 ± 4.54                        | 0.13                                                                    |
| Chit + Def + GaPP                | 50.4 ± 3.68                        | 26.4 ± 5.86                        | 0.39                                                                    |

**Table 1B. Patient VAS scores (mean and SEM) in each treatment group after FHF with Drill Out (FHF with DO)**

| FHF with DO, treatments received | VAS mean at Pre-Op 0 weeks | VAS mean at Post-Op 12 weeks visits | p-value of linear Mixed Model, longitudinal ANCOVA control Vs treatment |
|----------------------------------|----------------------------|-------------------------------------|-------------------------------------------------------------------------|
| no Rx Ctl                        | 36.2 ± 3.39                | 34.1 ± 3.5.3                        | -                                                                       |
| Chit                             | 31.1 ± 3.45                | 21.7 ± 3.91                         | 0.026                                                                   |
| Chit + Def                       | 35.2 ± 3.92                | 31.6 ± 4.24                         | 0.64                                                                    |
| Chit + Def + GaPP                | 36.2 ± 3.29                | 34.2 ± 3.77                         | 0.98                                                                    |

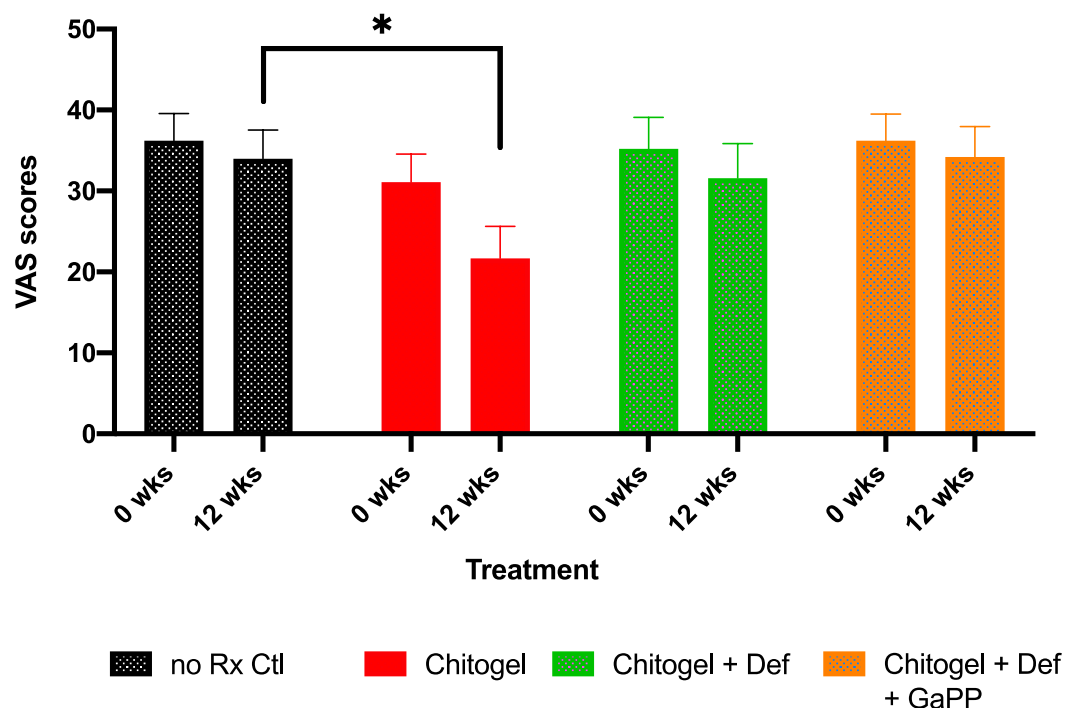
**Table 1C. Average percentage of Area of Frontal, Maxillary and Sphenoid Ostium after FHF with drill out at 12 weeks post treatment relative to ostium size at time=0**

| Frontal Drillout with Treatment | Mean Frontal ostium area in % at 12 wks PO | Mean Maxillary ostium area in % at 12 wks PO | Mean Sphenoid ostium area in % at 12 wks PO |
|---------------------------------|--------------------------------------------|----------------------------------------------|---------------------------------------------|
| DO no Rx Ctl                    | 74                                         | 89                                           | 77                                          |
| DO Chit                         | 96                                         | 100                                          | 80                                          |
| DO Chit +Def                    | 78                                         | 78                                           | 42                                          |
| DO Chit +Def + GAPP             | 90                                         | 95                                           | 82                                          |



**Figure 2, SNOT-22 responses before surgery (0 wks) and 12 weeks after FHF and frontal drillout (mean and SEM). WKS-weeks, Def-Deferiprone, GaPP-Gallium Protoporphyrin, \* p value<0.5**

Patient-reported VAS scores of facial pain/discomfort, bleeding, nasal obstruction, anterior and postnasal secretions and sense of smell after FHF with DO improved significantly over time across all groups ( $p<0.001$ ) and were significantly better in patients that received Chitogel than in control patients at the 12-week time point ( $p=0.026$ ) (Table 1B and Figure 3).



**Figure 3. VAS responses before surgery and at 12 weeks after FHF with Frontal Drillout, WKS-weeks, Def-Deferiprone, GaPP-Gallium Protoporphyrin, \* p value<0.5**

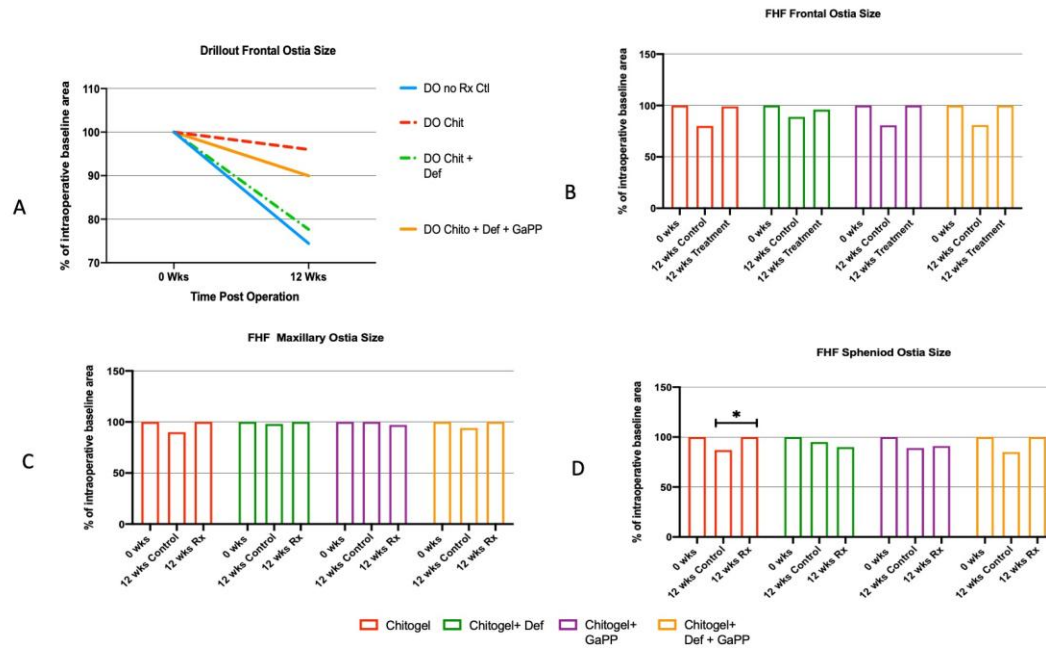
## **Comparison of surgeon reported ordinal scale of wound healing in the Full House FESS**

### **Group :**

Wound healing on both sides after FHF were assessed at each time point and untreated (no Rx Ctl) or treated with test product (Chitogel with or without Def and GaPP) was scored by 3 blinded surgeons (RV, YS, JL) using modified LK scores. Even though Chitogel treated sinuses appeared to heal better (Fig 4 & 5), there was no statistically significant difference in LKS for any of the test treatment groups when comparing the control untreated side with the test treatment side for each of those patient groups. Comparing LK scores between patient groups for the test treatment side only was not significantly different between the groups.

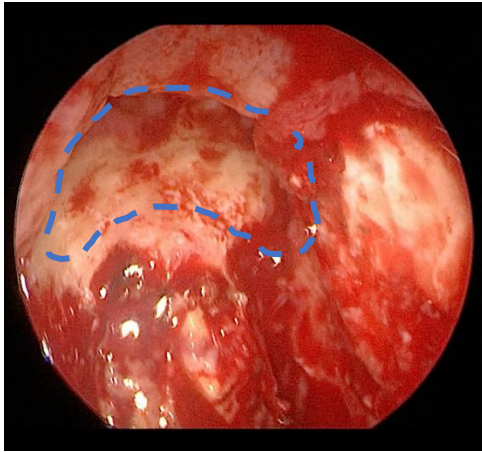
### **Comparison of sinus ostial size in the FHF + Drill out (DO) groups:**

The sinus ostial sizes were measured and compared at time = 0 (immediately after surgery) and at weeks 12 after surgery for each of the treatment groups (Fig 4 A & 5). In control patients (no treatment group), the mean frontal, maxillary and sphenoid ostium area reduced to 74, 89 and 77% of the original area measured at time=0. The mean ostium area for the Chitogel (96, 100 and 80% for the frontal, maxillary and sphenoid ostium respectively) as well as the Chitogel + Def + GaPP groups (90, 95 and 82% for the frontal, maxillary and sphenoid ostium respectively) were higher across all 3 sinuses compared to the no-treatment control group, however, those differences did not reach statistical significance (Table 1C ).

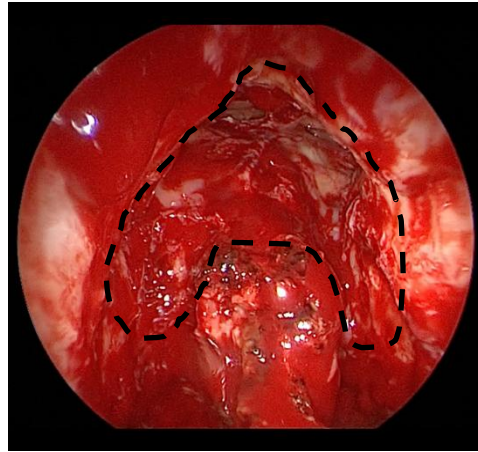


**Figure 4:** Ostium size of Frontal, Maxillary and Sphenoid sinuses at 12 weeks relative to 0 weeks (immediately after surgery) in patients receiving Full House FESS with Drill Out (A) or Full House FESS (B-D). Area of Frontal Ostium after FHF + Drill out (DO) in percentage at 12 weeks post treatment relative to ostium size at 0 weeks (A) for patients receiving no treatment (no Rx Ctl, blue line), Chitogel (Chit, red line), Chitogel + Def (Chit + Def, green line), Chitogel + Def + GaPP (Chit + Def + GaPP, orange line). Frontal (B), Maxillary (C) and Sphenoid (D) ostium size at the time of surgery (time= 0 weeks) and 12 weeks after surgery in control side and treatment side C. Maxillary Sinus ostium patency at surgery (0 weeks) and 12 weeks after surgery in control side and treatment side, Wilcoxon normalised to ostium size at time zero, , \*  $p < 0.05$

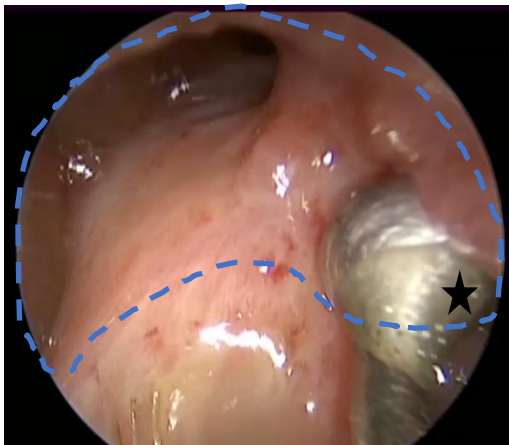




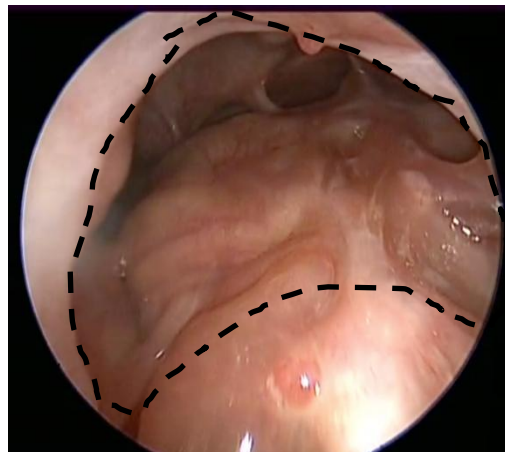
**A. DO no Rx Ctl, Frontal ostium Day 0**



**C. DO Chit, Frontal Ostium Day 0**



**B. DO no Rx Ctl, Frontal ostium,  
PO 12 weeks – Control**



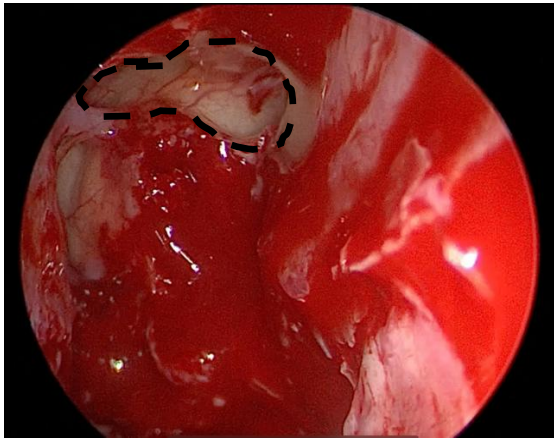
**D. DO Chit, Frontal Ostium PO 12 wks**

Figure 5, Endoscopic images of the frontal sinus ostium after FHF + Drill out at week 0 ( A&C) and 12 (B &D) with and without Chitogel™, showing a similar sized ostial opening with Chitogel treated sinus (d). \* calibrated measuring ball probe, broken line representing area of ostium , Day O – immediately after completion of surgery, PO – post operative , wks- weeks, FHF + Drillout Control - DO no Rx Ctl, FHF + Drillout with Chitogel alone -

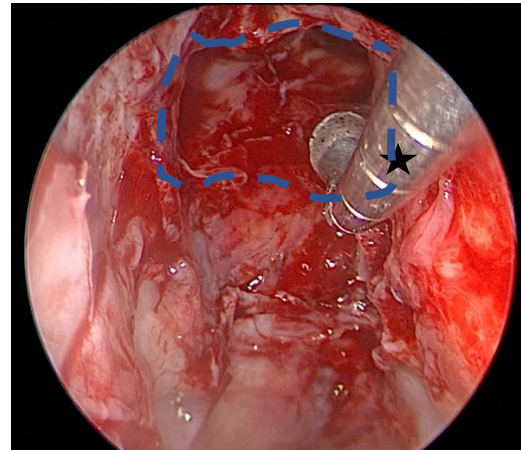
## DO Chit

**Comparison of sinus ostial size in the full house FESS (FHF) group:**

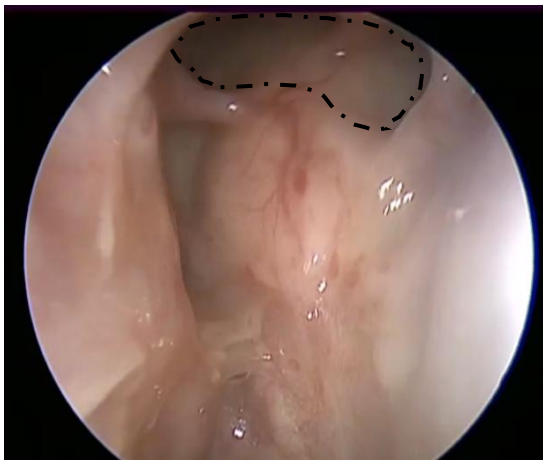
The sinus ostial sizes were measured and compared at time = 0 (immediately after surgery) and at weeks 12 after surgery for each of the treatment groups and for each of the sides (treated or not with test formulation). Normalised to the ostium size at time zero, the frontal ostium size in control sides (no treatment) reduced to <89% for all 4 treatment groups. In contrast, the mean frontal ostium size for all treatment sides reduced to maximum 96% for Chitogel + Def and was >99% for the Chitogel, Chitogel + GaPP and Chitogel + Def + GaPP treatment groups (Fig 4B & 6). However, those differences did not reach statistical significance. For the maxillary and sphenoid ostia sizes, there seemed to be more variability with the Chitogel and Chitogel + Def + GaPP treatment groups having ostia sizes that did not reduce at the 12 weeks' time point compared to ostia sizes at time zero (Figure 4C). The difference between ostium size of control and treatment side reached significance for the treatment with Chitogel where the sphenoid ostium size remained 100% compared to the ostia size at time zero and was significantly larger than the no-treatment control side at 87% ( $p<0.05$ )(Fig 4D).



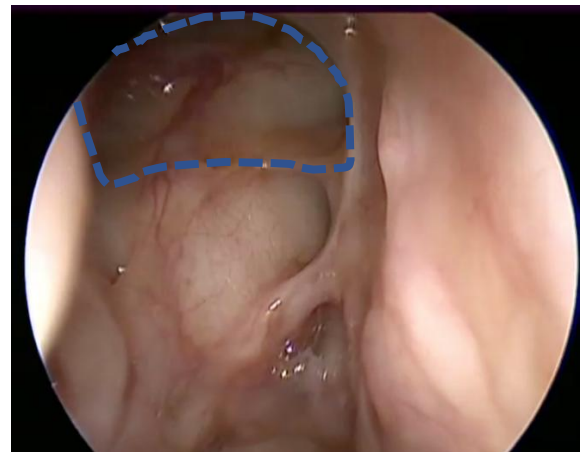
**a. FHF no Rx Ctl , R Frontal ostium  
Day 0**



**c. FHF Chit Left Frontal Day 0**



**d. FHF no Rx Ctl , L Frontal ostium,  
PO 12 wks**



**d. FHF Chit Left Frontal PO 12 weeks**

Figure 6 Endoscopic images of the frontal ostium FHF at week 0 ( a & c) and 12 ( b & d) with and without Chitogel™, showing well retained ostial opening with Chitogel treated sinus (d). \* calibrated measuring ball probe, broken line representing the area of ostium, Full House FESS

group -FHF, FHF Control - FHF no Rx Ctl, FHF Chitogel alone - FHF Chit

### **Blood tests for safety monitoring:**

All blood tests for markers of inflammation, serum ferritin levels and liver enzymes performed at 0 hours and 2 weeks post-operative visit were within normal limits and did not show any signs of toxicity.

### **Discussion:**

One of the biggest challenges in sinus surgery is preservation of the sinus ostia such that adequate aeration and drainage of the sinus can occur. In recent years the importance of effective topical therapy delivery in the post-operative period to the sinuses through these ostia has also been recognized<sup>443</sup>. In this study, we confirm the previously described benefits of Chitogel on preservation of sinus ostial size during the healing period<sup>46</sup>. We also show that adding Def and GaPP to Chitogel provides little additional benefit to using Chitogel alone. However, the safety of utilizing Def and GaPP in the post-operative environment was confirmed with no side effects from their use seen. The dose of the Def used in this study was higher than the dose used in a recently published paper where lower Def concentrations were shown to have higher anti-adhesive properties<sup>367</sup>. Further studies will need to be performed to better evaluate whether an inverse dose response can be seen for Def dosages lower than the 20 mM used in the present study. This study also demonstrates improved patient comfort and reduced symptom scores in patients who received Chitogel in the post-operative period as reflected by improvement in the quality of life and symptom scores (SNOT22 and VAS). Previous studies performed with Def and GaPP in combination<sup>85,96</sup> have shown synergistic

antibacterial activity. Given the numerous confounding factors affecting infective status, including the presence of raw mucosal surfaces and blood and well as the use of antibiotics and corticosteroids before, during and/or after surgery and in view that the primary outcome of this study was to evaluate safety and tolerability, whereas antibacterial effects of the treatments and Def-GaPP in particular was not comprehensively analysed. A further study specifically evaluating this aspect will be needed to determine the effect of Def-GaPP in Chitogel to reduce infection rates in the context of CRS and/or after surgery.

Sinus ostial patency post-surgery is dependent on creating the widest possible ostium with the maximal preservation of mucosa as well as limiting inflammation and blood clot within the ostium. Blood clot in itself creates inflammation so haemostasis is an important additional factor that needs to be addressed on completion of the surgery. One of the benefits of Chitogel over standard middle meatal stents is that it is applied into the sinus ostia thereby preventing blood clot formation within the ostium. Chitogel has been shown to have good haemostatic properties which help to prevent bleeding and blood clot formation. Furthermore, promoting mucosal healing by reducing inflammation and controlling bacterial activity with the prevention of fibroblast migration, has been demonstrated for Chitogel<sup>80,444</sup> and is thought to further improve ostial patency. The incorporation of Def and GaPP into Chitogel was intended to further improve wound healing in view of the known antibacterial, anti-inflammatory and anti-fibroblast migration properties of Def and GaPP<sup>22,23</sup>. Although both these agents were shown to have good safety and tolerability profiles, little additional benefit was seen in ostial preservation. A larger sample size is needed to comprehensively assess possible benefits with lower doses used in subsequent studies with good effect (paper under review) in terms of preservation of ostial size, mucosal healing and antibacterial effects.

**Conclusion:** Chitogel was shown to be an effective post-operative nasal dressing with improved patient comfort and symptoms after surgery. In addition, it was effective in maintaining sinus ostial size during healing. The addition of both Def and GaPP in the doses studied although safe showed no additional benefit.

**Supplementary table 1** Patient demography and randomization, M- male, F-female, FHF- full house functional endoscopic sinus surgery, DO-FHF+ drill out, A-Chitogel alone, B-Chitogel+Deferiporone(Def), C-Chitogel+Def+Gallium Protoporphyrin<sup>89</sup>, D-Citogel+GaPP in FHF, Non treatment control in DO, L-left side nasal cavity, R-right side nasal cavity, Pos-positive and neg-negative

| Clinical<br>Trial No: | Sex | Age | Surgery | Trial Arm | Randomization | Asthma |
|-----------------------|-----|-----|---------|-----------|---------------|--------|
| 1                     | M   | 28  | FHF     | C         | L             | neg    |
| 2                     | M   | 44  | FHF     | B         | R             | pos    |
| 3                     | M   | 29  | DO      | C         |               | neg    |
| 4                     | F   | 38  | FHF     | A         | L             | neg    |
| 5                     | F   | 23  | FHF     | A         | R             | neg    |
| 6                     | M   | 35  | FHF     | A         | L             | pos    |
| 7                     | F   | 54  | DO      | B         |               | pos    |
| 8                     | M   | 58  | FHF     | C         | R             | neg    |
| 9                     | M   | 79  | FHF     | B         | R             | neg    |
| 10                    | F   | 48  | DO      | B         |               | pos    |
| 11                    | F   | 36  | DO      | C         | R             | neg    |
| 12                    | M   | 49  | FHF     | C         | L             | neg    |

|    |   |    |     |   |   |     |
|----|---|----|-----|---|---|-----|
| 13 | M | 59 | DO  | D |   | pos |
| 14 | F | 67 | FHF | B | L | neg |
| 15 | M | 23 | FHF | C | R | neg |
| 16 | F | 66 | DO  | A |   | pos |
| 17 | M | 36 | DO  | A |   | neg |
| 18 | F | 61 | FHF | C | L | pos |
| 19 | F | 39 | FHF | B | L | pos |
| 20 | F | 50 | FHF | C | R | neg |
| 21 | F | 76 | FHF | B | L | neg |
| 22 | M | 36 | DO  | D |   | pos |
| 23 | M | 52 | FHF | B | R | neg |
| 24 | M | 78 | FHF | A | R | pos |
| 25 | M | 59 | FHF | A | R | neg |
| 26 | M | 39 | FHF | D | L | neg |
| 27 | M | 36 | DO  | C |   | pos |
| 28 | M | 79 | FHF | D | L | neg |
| 29 | F | 63 | FHF | D | R | neg |
| 30 | M | 38 | FHF | A | L | neg |
| 31 | M | 61 | FHF | D | L |     |
| 32 | M | 55 | FHF | D | R | neg |
| 33 | M | 30 | FHF | C | L | neg |
| 34 | F | 80 | FHF | C | L | neg |
| 35 | M | 50 | FHF | B | R | neg |
| 36 | M | 60 | FHF | D | R | neg |

Modifying wound healing and PO outcome

|    |   |    |     |   |   |     |
|----|---|----|-----|---|---|-----|
| 37 | F | 33 | DO  | D |   | neg |
| 38 | M | 69 | FHF | C | R | neg |
| 39 | M | 56 | FHF | D | L | neg |
| 40 | F | 51 | FHF | C | L | neg |
| 41 | F | 43 | DO  | C |   | neg |
| 42 | F | 65 | FHF | D | R | neg |
| 43 | F | 29 | DO  | B |   | pos |
| 44 | F | 68 | FHF | A | L | pos |
| 45 | F | 51 | FHF | B | R | pos |
| 46 | F | 55 | DO  | B |   | pos |
| 47 | M | 29 | FHF | A | R | pos |
| 48 | F | 69 | FHF | B | L | pos |
| 49 | F | 51 | DO  | D |   | pos |
| 50 | M | 49 | DO  | D |   | pos |
| 51 | F | 49 | DO  | A |   | pos |
| 52 | M | 66 | DO  | B |   | neg |
| 53 | F | 72 | FHF | A | R | neg |
| 54 | M | 56 | FHF | A | L | neg |
| 55 | M | 64 | DO  | C |   | neg |
| 56 | M | 56 | DO  | A |   |     |
| 57 | M | 41 | DO  | D |   | pos |
| 58 | M | 48 | DO  | A |   | pos |
| 59 | F | 47 | DO  | A |   | pos |
| 60 | M | 77 | DO  | D |   | neg |



Modifying wound healing and PO outcome

|    |   |    |     |   |   |     |
|----|---|----|-----|---|---|-----|
| 61 | F | 82 | FHF | D | L | neg |
| 62 | M | 62 | FHF | D | R | pos |
| 63 | F | 55 | DO  | A |   | neg |
| 64 | F | 52 | DO  | C |   | pos |
| 65 | M | 74 | DO  | C |   | neg |
| 66 | M | 69 | FHF | B | L | neg |
| 67 | F | 60 | DO  | A |   |     |
| 68 | F | 25 | DO  | B |   | pos |
| 69 | F | 66 | DO  | B |   | pos |
| 70 | M | 59 | DO  | B |   | pos |
| 71 | M | 61 | DO  | C |   | neg |
| 72 | M | 23 | DO  | D |   | pos |
| 73 | M | 47 | DO  | D |   | pos |
| 74 | M | 51 | DO  | C |   | neg |
| 75 | M | 46 | DO  | A |   | neg |
| 76 | M | 52 | DO  | A |   | pos |
| 77 | M | 60 | DO  | B |   | pos |
| 78 | M | 66 | DO  | C |   | neg |
| 79 | M | 50 | DO  | C |   | neg |

## 7 Chitogel with Def - GaPP in Abdominal Surgery:

**Summary:** Adhesion in the abdominal cavity after surgery is a result of natural process of wound healing that is either excessive or abnormal. The unwanted consequences of adhesion are Pain, Intestinal obstruction or infertility, with an overall burden of 2.3 billion on health expenditure<sup>302</sup>, with immeasurable human suffering and cost. Due to this there has been a constant effort by surgeons and researchers to innovate surgical techniques and materials that could prevent adhesion formation<sup>306</sup>. Wound healing in the abdomen after surgery is unique, after peritoneal injury there is a rapid fibrin matrix formation within 30 minutes of haemostasis, and there is fibroblast migration within 2 hrs to 1 week<sup>445</sup>. There is a need for anti-adhesive products or barriers which will be able to regulate the wound healing, of which haemostasis is the primary step followed by regulation of fibroblast activity. Associated with this is the accentuation of adhesion formation in the presence of infection due to contamination of the wound site either due to surgery or post-surgical complication<sup>363</sup>. Hence, there is search for a product which has excellent anti-adhesive property and potent anti-microbial activity. Among many products in use, barrier system is the most effective, and there is no universally acceptable product available, which has led to search for an ideal barrier system. The team at TQEH have done preliminary experiments and have optimized murine and porcine models of abdominal surgery<sup>332,362</sup>. The ideal volume, composition and consistency of the Chitogel for intra-abdominal application was determined in these models. There is a further need to develop treatment regimens along with this for conditions such as adhesions due to Intra-abdominal infections. One of the challenges in determining the usefulness of the anti-adhesive barrier effect is to have an animal model that will be mimic the human intra-abdominal condition and also be safely replicable in animals. We propose to use Kaolin as an adhesion inducing agent over a surgical gauze induced injury by rubbing over the caecum until bleeding. This novel technique is first of its kind and after validation we

proceeded to use Chitogel with various combination and concentrations of Deferiprone and Gallium Protoporphyrin to determine the safe and effective combination and their dose.

### **7.1.1 A Novel Rat Model to Test Intra-Abdominal Anti-Adhesive Therapy**

Conducted in the Department of Otolaryngology – Head and Neck Surgery

The University of Adelaide, Adelaide, Australia,

Financial assistance provided by The University of Adelaide and The Hospital Research

Foundation under Australian Government Research Training Program Scholarship



# A Novel Rat Model to Test Intra-Abdominal Anti-adhesive Therapy

Rajan Sundaresan VEDIAPPAN<sup>1</sup>, Catherine Bennett<sup>1</sup>, Ahmed Bassiouni<sup>1</sup>, Matthew Smith<sup>2</sup>, John Finnie<sup>3</sup>, Markus Trochsler<sup>4</sup>, Alkis J. Psaltis<sup>1</sup>, Sarah Vreugde<sup>1</sup> and Peter J. Wormald<sup>1\*</sup>

<sup>1</sup> Department of Surgery - Otolaryngology Head and Neck Surgery, The University of Adelaide, Adelaide, SA, Australia, <sup>2</sup> The Queen Elizabeth Hospital, Animal Experiment Unit, Adelaide, SA, Australia, <sup>3</sup> SA Pathology and Adelaide Medical School, The University of Adelaide, Adelaide, SA, Australia, <sup>4</sup> Department of Surgery, The University of Adelaide, Adelaide, SA, Australia

## OPEN ACCESS

### Edited by:

René H. Fortelny,  
Wilhelminenspital, Austria

### Reviewed by:

John Griniatsos,  
Laiko General Hospital of  
Athens, Greece  
Premkumar Balachandran,  
Apollo Speciality Hospitals, India

### \*Correspondence:

Peter J. Wormald  
peterj.wormald@adelaide.edu.au;  
pjwormald@gmail.com

### Specialty section:

This article was submitted to  
Visceral Surgery,  
a section of the journal  
Frontiers in Surgery

**Received:** 20 May 2019

**Accepted:** 06 March 2020

**Published:** 08 April 2020

### Citation:

Vediappan RS, Bennett C,  
Bassiouni A, Smith M, Finnie J,  
Trochsler M, Psaltis AJ, Vreugde S  
and Wormald PJ (2020) A Novel Rat  
Model to Test Intra-Abdominal  
Anti-adhesive Therapy.  
Front. Surg. 7:12.  
doi: 10.3389/fsurg.2020.00012

**Background:** Adhesion formation after abdominal surgery is considered almost inevitable and a major cause of morbidity. Novel treatments have been proposed, however there is a lack of suitable small animal models for pre-clinical evaluation, mainly due to inconsistency in adhesion formation in positive control animals. Here, we propose a new rat model of abdominal adhesions using Kaolin as the adhesion-inducing agent at an optimized dosage for testing newer agents in respect to their anti-adhesive property.

**Materials and Methods:** Twenty-five adult (8–10 week old) male Wistar albino rats underwent midline laparotomy and caecal abrasion and were randomized to receive topical applications of normal saline or different concentrations and volumes of a Kaolin-based formulation. At day 14 rats were humanely killed, and adhesions graded macroscopically by an investigator blinded to the treatment groups, using pre-determined adhesion scores and microscopically using histopathology.

**Results:** Kaolin at 0.005 g/mL caused consistent adhesions without compromising rat viability. At higher doses significant morbidity and mortality was observed in the animals treated.

**Conclusions:** Kaolin induced adhesion in a rat abdominal surgery model is reliable and can be safely used to test the efficacy of novel anti-adhesive formulations to prevent intra-abdominal adhesions.

**Keywords:** Kaolin, abdominal adhesion, animal model, fibrosis, anti-adhesive agent

## INTRODUCTION

Scarring or fibrosis is an inevitable manifestation of the wound healing process in the human body after surgery. This often results in undesirable outcomes. Scarring after abdominal surgery often results in the formation of adhesions where scar tissue connects organs with each other, often resulting in post-surgical morbidity. Around 7 million open abdominal surgeries occur each year in the US and Europe with adhesions estimated in up to 90% of cases, costing the USA health care system \$USD 2.3 billion annually (1). Postsurgical adhesions are the largest single cause of intestinal obstruction with a mortality rate of 10% and can also contribute to female infertility (2–5). Numerous strategies have been recommended to prevent peritoneal adhesions; however, none are widely adopted due to poor efficacy or risk of adverse events (6). It is essential to have an

## Statement of Authorship

|                     |                                                                                                                                                                                                                                                |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title of Paper      | A Novel Rat Model to Test Intra-Abdominal Anti-Adhesive Therapy                                                                                                                                                                                |
| Publication Status  | <input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication<br><input type="checkbox"/> Submitted for Publication<br><input type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Published in Journal Frontiers in Surgery – Visceral Surgery section on 08.04.2020                                                                                                                                                             |

### Principal Author

|                                      |                                                                                                                                                                                                                                                                                                |      |            |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------|
| Name of Principal Author (Candidate) | Vediappan, Rajan Sundaresan                                                                                                                                                                                                                                                                    |      |            |
| Contribution to the Paper            | Conduct of study, data collection, sample processing, Image analysis, statistical analysis and manuscript preparation                                                                                                                                                                          |      |            |
| Overall percentage (%)               | 75 %                                                                                                                                                                                                                                                                                           |      |            |
| Certification:                       | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |      |            |
| Signature                            |                                                                                                                                                                                                                                                                                                | Date | 20.05.2019 |

### Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above)
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

|                           |                                                                                                           |      |          |
|---------------------------|-----------------------------------------------------------------------------------------------------------|------|----------|
| Name of the Author        | Bennett, Catherine                                                                                        |      |          |
| Contribution to the Paper | Conduct of study, sample processing, Image analysis, statistical analysis and manuscript preparation- 10% |      |          |
| Signature                 |                                                                                                           | Date | 20.05.19 |
| Name of the Author        | Bassiouni, Ahmed                                                                                          |      |          |
| Contribution to the Paper | Data Analysis, Interpretation of results and drafting of Paper - 1%                                       |      |          |

|                           |                                                                                                                                                  |      |          |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|------|----------|
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |
| Name of the Author        | Smith, Matthew                                                                                                                                   |      |          |
| Contribution to the Paper | Conduct of study and Data collection - 1%                                                                                                        |      |          |
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |
| Name of the Author        | Finnie, John                                                                                                                                     |      |          |
| Contribution to the Paper | Conduct of study, sample processing, Image analysis, statistical analysis and manuscript preparation- 1%                                         |      |          |
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |
| Name of the Author        | Trochsler, Markus                                                                                                                                |      |          |
| Contribution to the Paper | Image analysis, statistical analysis and manuscript preparation - 1%                                                                             |      |          |
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |
| Name of the Author        | Psaltis, Alkis J.                                                                                                                                |      |          |
| Contribution to the Paper | Critical Intellectual input for manuscript preparation and approval for submission - 1 %                                                         |      |          |
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |
| Name of the Author        | Vreugde, Sarah                                                                                                                                   |      |          |
| Contribution to the Paper | Design Conceptualization, Interpretation of data, critical intellectual input for manuscript preparation and approval for submission - 5 %       |      |          |
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |
| Name of the Author        | Wormald, Peter-John                                                                                                                              |      |          |
| Contribution to the Paper | Design Conceptualization, Interpretation of data, critical intellectual input for manuscript preparation and final approval for submission - 5 % |      |          |
| Signature                 |                                                                                                                                                  | Date | 20.05.19 |

## **A novel rat model to test intra-abdominal anti-adhesive therapy**

Rajan Sundaresan VEDIAPPAN<sup>1</sup>, Catherine BENNETT<sup>1</sup>, Matthew SMITH<sup>3</sup>, Ahmed BASSIOUNI<sup>1</sup>, John FINNIE<sup>2</sup>, Markus TROSCHLER<sup>4</sup>, Alkis PSALTIS<sup>1</sup>, Sarah VREUGDE<sup>1</sup>, Wormald PJ<sup>1</sup>

<sup>1</sup>Department of Otolaryngology Head and Neck Surgery, The University of Adelaide

<sup>2</sup>SA Pathology and Adelaide Medical School, University of Adelaide, Adelaide

<sup>3</sup>The Queen Elizabeth Hospital, Adelaide

<sup>4</sup>Department of Surgery, The University of Adelaide

Conflict of Interest: Nil

Corresponding Author:

Professor Peter John Wormald  
The University of Adelaide  
Department of Otolaryngology, Head & Neck Surgery  
The Queen Elizabeth Hospital  
28, Woodville Road, Woodville south-5011  
Adelaide, South Australia  
Contact: peterj.wormald@adelaide.edu.au  
Ph: (+61 8) 8222 7158 Fax: (+61 8) 8222 7419

Author Contributions:

RSV, CB, MS, JF MT & AB were involved in the conduct of the study, collection of data and interpretation of the result with drafting and editing the manuscript.

PJW, SV & AP were involved in designing the study, supervision, Interpretation of data, drafting and editing the manuscript and final permission for submission.

Abstract:



**Background:** Adhesion formation after abdominal surgery is considered almost inevitable and a major cause of morbidity. Some novel treatments have been proposed however, there is a lack of suitable small animal models for pre-clinical evaluation, mainly due to inconsistency in adhesion formation in positive control animals. Here, we propose a new rat model of abdominal adhesions using Kaolin as adhesion-inducing agent at an optimised dosage.

**Materials and Methods.** Twenty-five adult (8-10week old) male Wistar albino rats underwent midline laparotomy and caecal abrasion and were randomized to receive topical applications of normal saline or different concentrations and volumes of a Kaolin-based formulation. At day 14 rats were euthanized, and adhesions graded macroscopically by an investigator blinded to the treatment groups, using pre-determined adhesion scores and microscopically using histopathology.

**Results:** Kaolin at 0.005 g/mL caused consistent adhesions without compromising rat viability. At higher doses significant morbidity and mortality was observed in the animals treated.

**Conclusions:** Kaolin induced adhesion in a rat abdominal surgery model is reliable and can be safely used to test the efficacy of novel anti-adhesive formulations to prevent intra-abdominal adhesions.

**Keywords:** Kaolin, abdominal adhesion, animal model, fibrosis, anti-adhesive agent

## **Introduction:**

Scarring or fibrosis is an inevitable manifestation of the wound healing process in the human body after surgery resulting in undesirable outcomes. Scarring after abdominal surgery often results in the formation of adhesions where scar tissue connects organs with each other, often resulting in post-surgical morbidity. Around 7 million open abdominal surgeries occur each year in the US and Europe <sup>446</sup> with adhesions presenting in up to 90% of cases, costing the health care system \$USD 2.3 billion annually <sup>302</sup>. Postsurgical adhesions are the largest single cause of intestinal obstruction with a mortality rate of 10% and can cause female infertility <sup>274,447</sup>. Numerous strategies have been recommended to prevent peritoneal adhesions however, none of those are widely adopted due to poor efficacy or risk of adverse events <sup>305</sup>. It is essential to have an animal model that could be used to test novel anti-adhesive strategies in abdominal surgery or to test substances that could prevent adhesions. Different models have been proposed but a recurring problem is the high variability of adhesions in positive control animals and a better animal model is required. Kaolin is known to induce inflammation and foreign body reactions and has been used to induce adhesions in animal models, especially in pulmonary fibrosis <sup>448,449</sup>, hepatic fibrosis <sup>450</sup>, and subarachnoid dural adhesion clinical models <sup>451</sup>. This study tested the dose-dependent effects of kaolin to induce adhesions in a rat colon abrasion model.

## **Materials & Methods:**

The University of Adelaide and Central Adelaide Local Health Network/SA Pathology Animal Ethics Committees (AEC) approved the study to be conducted at The Queen Elizabeth Hospital Experimental Surgical Suite (The University of Adelaide AEC M-2017-061 and CALHN/SA Pathology AEC 25-17).

### **Animals and materials:**

Male Wistar albino rats were purchased from Laboratory Animal Services

Medical School (The University of Adelaide, SA, Australia), 8 to 10 weeks old, with an average weight between 350-500 grams. Rats were housed 1 week prior to surgery under standard laboratory conditions (temperature  $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , humidity  $55\% \pm 10\%$ , 12: 12-hour light-dark-cycle). Rats were housed in groups of 3 per cage and food and water were provided in a standard manner. Kaolin (Aluminium silicate Hydroxide,  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ ) was purchased from Sigma-Aldrich, St. Louis, Missouri, United States.

### **Surgical procedure:**

Surgical procedures were performed by the same surgeons (RSV, CB) and a maximum group size of five animals per day was used to ensure close monitoring during the immediate post-operative period. Anaesthesia was achieved using a sealed chamber to deliver 2%-3% Isoflurane after which the animal was positioned supine for surgery and anaesthesia maintained with isoflurane over an open mask. Analgesia was provided preoperatively by subcutaneous injection of Buprenorphine (0.05mg/kg) and post-operative 8 hourly for 48 hours. The surgery was conducted in aseptic manner and a prophylactic dosage of broad-spectrum antibiotic in the form of Amoxicillin Clavulanic acid 5 mg/kg (Clavulox<sup>\*</sup> Zoetis Australia, Rhodes, NSW, Australia) was also administered via subcutaneous injection.

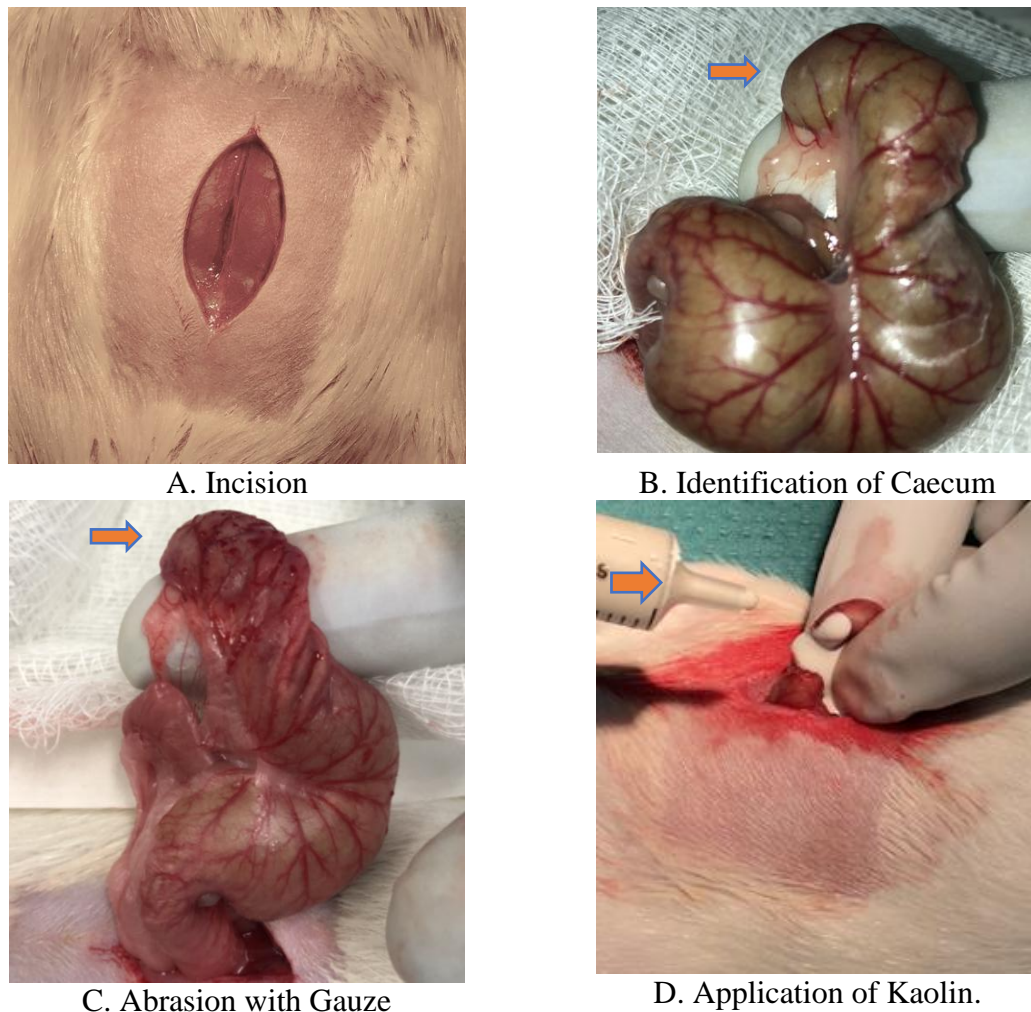


Figure1: A. Incision over the Rat abdominal wall after preparation, B. Identification of Caecum (orange arrow), C. Abrasion over the caecum with gauze till bleeding spots appear, D. Application of Kaolin over the abrasion/enterotomy,

Rats then underwent a laparotomy and a colon abrasion<sup>332</sup> or a colon abrasion with enterotomy. Briefly, the abdomen was shaved and prepared with alcohol and after drying, a 3 cm laparotomy was performed to gain access to the abdominal cavity (Fig 1A). In the caecal abrasion group, the caecum was delivered (Fig.1B) and kept moist with saline-soaked gauze whilst a dry gauze was used to rub the caecum repeatedly until sub-serosal bleeding occurred over an area of 1 cm<sup>2</sup> (Fig. 1C). The caecum was then returned to the abdomen and the

abdominal wall closed in layers with a 3-0Polyglactin suture. Prior to the placement of the final abdominal closure suture, rats were randomized to receive the following treatments:

- (1) 4 mL normal saline, n=5
- (2) 4 mL 0.25g/mL mixture of Kaolin/normal saline, n=5
- (3) 2 mL 0.1g/mL mixture of Kaolin/normal saline, n=5
- (4) 2mL 0.005g/mL mixture of Kaolin/normal saline, n=5

The operation was limited to <20 mins each rat so as to avoid air drying of the organs.

In a second stage, we used a colon abrasion with enterotomy model (n=5) to simulate a colon resection with anastomosis performed at a different site on the caecum to the abrasion. Rats underwent a laparotomy as above followed by a caecum incision to create a full thickness enterotomy over a length of 1 cm away from the abrasion site. The enterotomy defect was then closed with a continuous 4-0 PDS suture (resorbable, monofilament) and the repair leak tested with a simple pressure test. 2 ml 0.005g/mL Kaolin in saline was instilled over the abrasion (Fig.1D) and sutured site before closure of the abdominal wall. The rats in this group were monitored for 3 weeks as part of the larger experiment protocol.

### **Postoperative monitoring**

Post-surgery, the animals were housed individually in separate cages. Animals were monitored postoperatively 8-hourly for the first 48 hours to observe their weight, behaviour, physical wellbeing and appearance by using the Clinical Record Sheet, as approved by AEC. Distress scores higher than 6 or weight loss greater than 15% required that animals be humanely killed.

### **Outcome measures:**

The animals were humanely killed on post-operative day 14 and scored based on the presence and severity of adhesions using a previously validated adhesion scoring system as in Table 1

(36). The score takes into account the number, strength and distribution of adhesions formed. Pictures were taken by iPhone8 12mp *f*/1.8 aperture camera and also evaluated by a blinded observer.

Table 1. Adhesion Scoring Scheme

| Adhesion Scoring Scheme |                                                                    |
|-------------------------|--------------------------------------------------------------------|
| Adhesion Scoring Scheme | Score Description                                                  |
| 0                       | No adhesions                                                       |
| 1                       | Thin filmy adhesions                                               |
| 2                       | More than one thin adhesion                                        |
| 3                       | Thick adhesion with focal point                                    |
| 4                       | Thick adhesion with planar attachment                              |
| 5                       | Very thick vascularized adhesions or more than one planar adhesion |

### Histology:

The caecum, and adhesions between the caecal adventitia and adherent, adjacent intestinal serosal surfaces, and between the adventitial aspect of the caecum and the parietal peritoneum of the abdominal wall, were collected and immersion-fixed in 10% neutral buffered formalin. These tissues were then paraffin-embedded, cut at 6µm, and stained with haematoxylin and eosin (H&E). Duplicate sections were also stained by the Masson's trichrome technique to demonstrate collagen deposition in fibrous adhesions.

**Statistical Analysis:** All statistics were performed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria) through the Jupyter notebook interface. The R package "MASS"<sup>442</sup> was used for ordinal regression. The "polr" function from MASS was

used to fit a proportional odds logistic regression model for the ordinal outcome variable (the adhesion score as scored by the primary surgeons). A Likelihood ratio test (using the R function "anova") was used to compare the model with a null ordinal regression model. The means of the ordinal response (interpreted as a numeric value from 1 to the number of classes) were calculated and post-hoc pairwise contrasts for each pair of levels of the treatment variable were compared using the "emmeans" package.<sup>442</sup>. Statistical significance was taken at the traditional 0.05 level.

## **Results:**

### **Adhesion Scores**

Control rats receiving colon abrasion and saline and had variable adhesion scores with a mean adhesion score of 1 (SD 1) and 2/5 having an adhesion score of 0 (no adhesions) (Figure 2A).

Four rats died in the treatment groups with high Kaolin doses, 2 in the group treated with 4ml 0.25 g/ml and 2 in the group receiving 2ml 0.1 g/ml Kaolin (Figures 2B,C). Post-mortem evaluation showed severe adhesions with complications of intestinal obstruction, thought to be the likely cause of demise. The remaining rats which lasted the full 14 days showed mean adhesion grades of 4 (SD 0.44) (Figure 3A) and 4.6 (SD 0.6324) (Figure 3B) for 4ml 0.25 and 2ml 0.1 g/mL respectively.



a. Saline (Grade 0)



b. Kaolin 0.025g/ml (Grade 5)



c. Kaolin 0.1g/ml (Grade 5)



d. Kaolin 0.005g/ml (Grade 3)

Figure 2: a. Post euthanasia Caecum saline treatment showing minimal or no adhesion, b. Post euthanasia Caecum Kaolin 0.025g/ml showing Grade 5 adhesion, c. Post euthanasia Caecum Kaolin 0.1g/ml treatment showing Grade 5 adhesion, d. Post euthanasia Caecum Kaolin 0.005g/ml treatment showing Grade 3 adhesion.



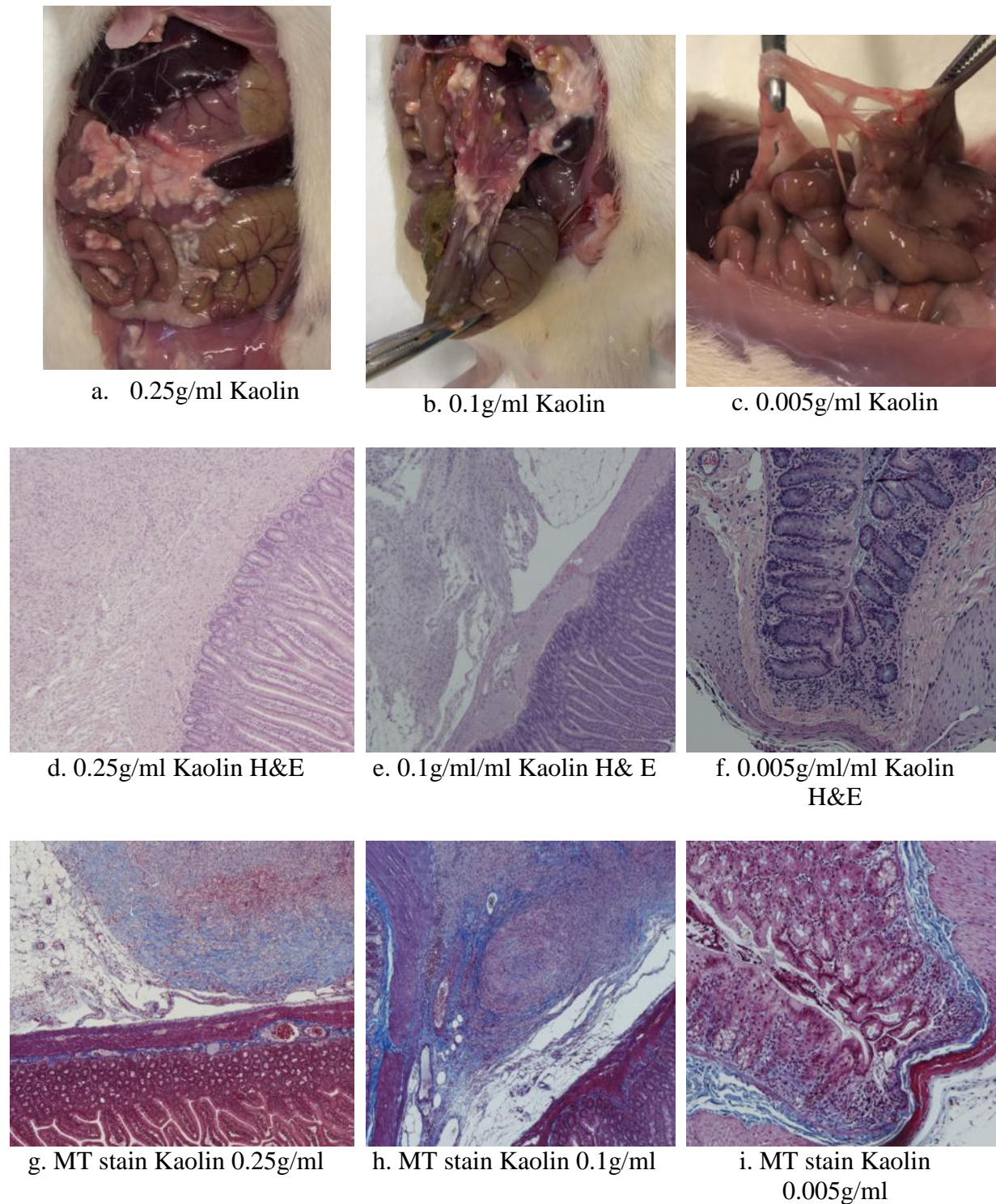


Figure 3: | (A) Macroscopic (A–C) and histopathological (D–I) evaluation of abdominal cavity of Rats treated with various concentrations of Kaolin. L, liver.C, caecum; A, adhesion. (A) 0.25 g/ml Kaolin causing very thick vascularized adhesions or more than one planar adhesion (Grade 5), (B) 0.1 g/ml Kaolin causing very thick adhesions with planar adhesion

(Grade 4), (C) 0.005 g/ml Kaolin causing thick adhesion with focal point (red arrow) (Grade 3) (D–I). Histopathology of rat caecum, 4X magnification using Haematoxylin & Eosin staining (D–F) and Masson's Trichrome staining (G–I). 0.25 g/ml Kaolin treatment showing thick adhesions and polymorphonuclear cell infiltrates (D) with disorderly and dense collagen deposition (G). 0.1 g/ml Kaolin with polymorphonuclear cell infiltrates and foreign body reaction (E) and disorderly and dense collagen deposition (H). 0.005 g/ml Kaolin with minimal polymorphonuclear cells (F) and orderly and light collagen deposition (I).

Five rats received 2ml 0.005 g/mL of Kaolin. These rats tolerated the procedure well with no significant morbidity or mortality at the end of 14 days recovery period. The resultant adhesions were mean grade 3.4 SD 0.54 (Figure 2D). The grade of adhesions was significantly greater in the 0.005 g/mL Kaolin treated rats compared to saline treated rats ( $p < 0.0001$ ). Similarly, the abrasion with enterotomy group, treated with Kaolin 0.005 g/ml showed much thicker and vascularized adhesions consistently over the enterotomy site in comparison to the abrasion site. These rats had mean adhesion grade of 4(SD 0.816) and was significantly higher than the Kaolin 0.005 g/ml treated abrasion. alone model with adhesion grade 3.4(SD0.54) ( $p < 0.0001$ ).

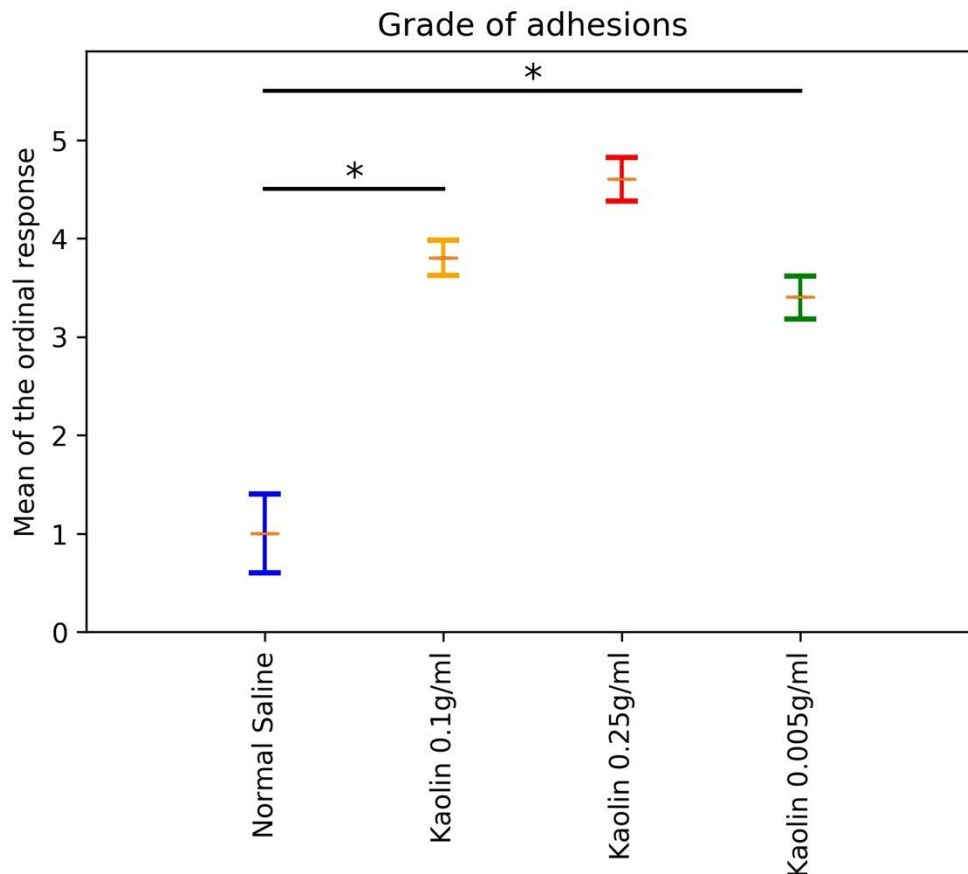
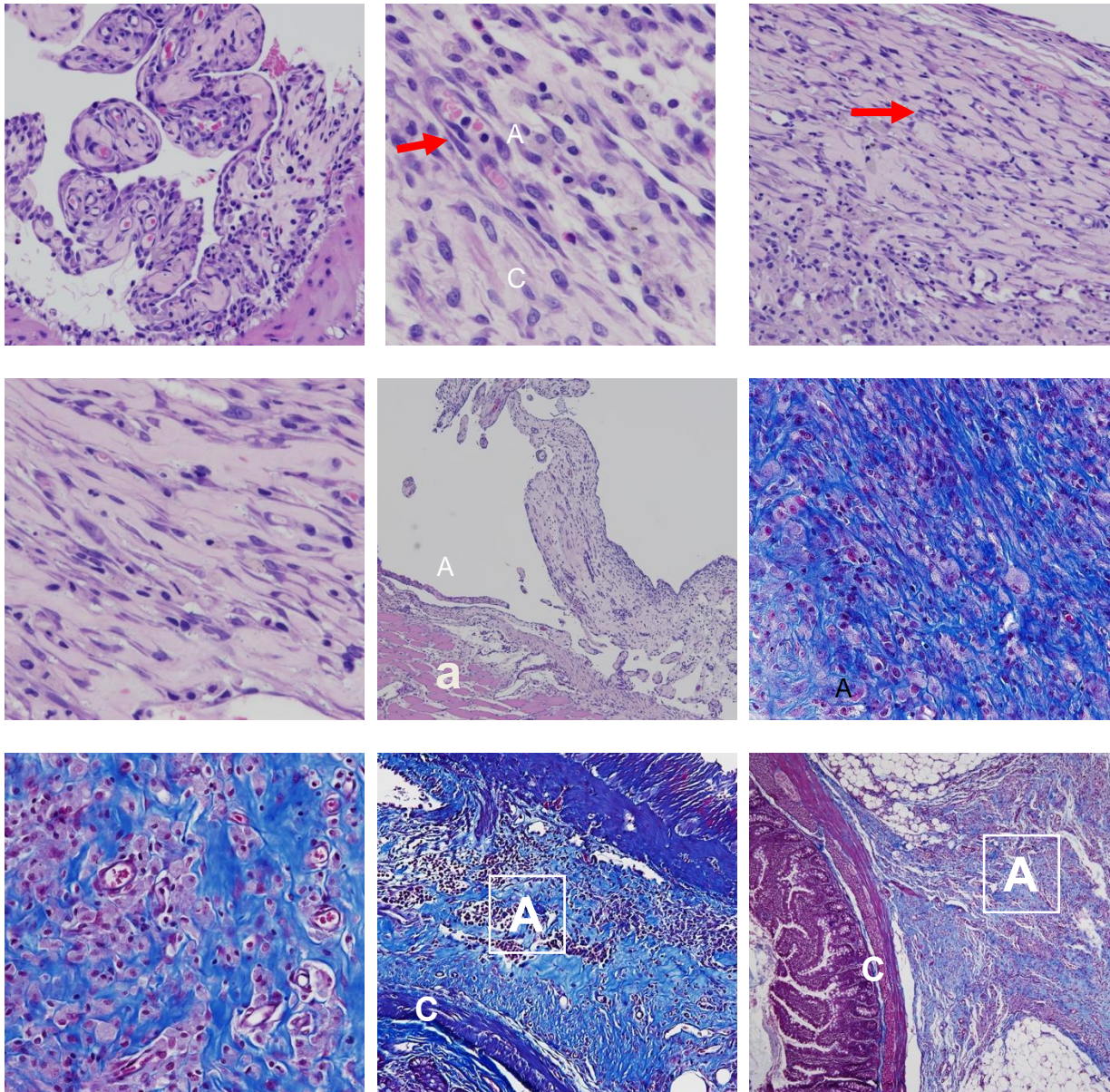


FIGURE 4 | Laparotomy Adhesion score: Bar plot showing different dosages of Kaolin induced adhesion against Ordinal scale in Rats undergone laparotomy and abrasion. The Normal saline treated Rats had minimal, inconsistent adhesion. Rats treated with Kaolin 0.1 g/ml and 0.25 g/ml induced a high grade of adhesion. Rats treated with 0.005 g/ml of Kaolin showed a consistent grade of adhesion between

### Histopathology:

Microscopic analysis of the various grades of adhesions formed in the presence of Kaolin showed classical foreign body (FB) reaction with granular activity at the epicentre of inflammation (Supplementary Figure 1-I) which was not seen in the saline treated caecum.





Supplementary Figure 1 | Illustrates the temporal development of adhesion.(I) Formation of an active adhesion projecting from the caecal adventitial surface and comprised of fibrovascular granulation tissue and collagen deposition (arrow)H&E. (II) Higher power view of a similar adhesion to that shown in this figure. There is robust fibrovascular proliferation and invading macrophages containing phagocytosed administered exogenous material (arrow) H&E. (III) An early adhesion showing active fibroblastic proliferation, with loosely arranged collagen fibrils evident. Numerous micro-vessels are present (arrow) H&E. (IV)

More mature adhesions showing abundant collagen deposition. H&E. (V) Adhesion projecting from the abdominal wall (abdominal wall) H&E. (VI,VII) Adhesion composed of numerous bundles of collagenous connective tissue, admixed with invading macrophages (arrows). Masson's trichrome. (VIII) Well-developed fibrous adhesion between two loops of bowel. Masson's trichrome (A in box Adhesion, C-caecal serosa wall). (IX) Diffuse adhesion projecting from the caecal serosa wall.

The FB reaction was in the form of numerous invading macrophages(Supplementary Figure 1III), which contained phagocytosed kaolin, with active fibrovascular granulation tissue formation with numerous proliferating fibroblasts and supportive micro vessels(Supplementary Figure 1II). There were mature adhesions with abundant compact collagen and fewer fibroblasts in the 0.1 and 0.25 g/ml kaolin treated rats. The presence of adhesions was predominantly confined to the abrasion site and one rat to the abdominal wall at the suture site (Supplementary Figure 1V). Masson's trichrome stain (MT stain) demonstrated a clear pattern of adhesion formation due to fibroblastic activity at various stages(Supplementary Figures 1VI–IX). The adhesions from threats that were treated with higher concentrations of Kaolin(0.25 and 0.1 g/ml) showed a very irregular pattern of collagen distribution (Supplementary Figure 1VI) compared to the uniform nature in the lower dosage group of Kaolin 0.005 g/ml(Supplementary Figure 1IX).

### **Discussion:**

This study showed that Kaolin at low dosage of 0.005g/ml induced thick peritoneal adhesions in the abdominal cavity, consistent and non-lethal in a rat colon abrasion and colon enterotomy model. This model produced consistent moderate to severe grade adhesions with a uniform distribution of collagen fibres on microscopic examination.

Creating an animal model for abdominal adhesions with consistent, reliable and reproducible findings for the positive control is a challenge. Unlike humans, where adhesions are almost inevitable after abdominal surgery, in the rat model, no or only low-grade adhesions are commonly found after laparotomy alone. Also, in this study, no or limited adhesions were found in the saline control animals. Several types of animal models have been used, small (mice, rat and rabbit) and large (sheep, pig, monkey and horse)<sup>368</sup>. Models do stimulate adhesion-formation in different ways, including colon and side wall abrasion, crushing, desiccation, incision, excision, electrocautery, laser injury, thermal injury, chemical injury, radiation injury, and foreign body-tissue irritation<sup>445</sup>. However, the usefulness of those models is hampered by the variability of adhesion formation in the positive control animals. This reduces the power of those studies increasing the number of animals that is required to test the anti-adhesive properties of test compounds and takes a longer period to replicate. Indeed, the strength of a model lies in the ability to replicate a similar injury process as in human conditions producing similar uniform non-lethal forms of adhesions in positive control animals. Kraemer et al<sup>452</sup> compared 5 different types of injury models and demonstrated good adhesion formation but they were performed on the parietal wall of the abdomen which does not mimic the laparotomy model and does not cause the serosal or mesothelial injury. diZeerga et al describes that clean-cut incisional wounds are not enough to stimulate fibrin deposition and in contrast, cautery and thermal injury causes excessive tissue necrosis with formation of mature fibrotic bands after more than 21 days<sup>445</sup>. Özel et al<sup>453</sup> discusses the chemical injury model using alcohol and iodine which are inherently disinfectants and are not suitable for an infective (enterotomy) model. Hence a chemical which is potent enough to create a foreign body reaction at the site of mechanical injury caused by abrasion and limited in its role as a general irritant is ideal. Kaolin or commonly

called ‘chalk’, is a mixture of different minerals and is a naturally occurring aluminium silicate mineral derived from clay. It contains quartz, mica, feldspar, iolite and montmorillonite. Kaolin is used in paper production, in paints, rubber, plastic, ceramic, chemical, pharmaceutical and cosmetic industries<sup>454</sup>. Jaurand and Pairon<sup>455</sup> in 1990 studied the interaction of Kaolin with cell lines and found a variety of membrane interactions and metabolic impairments. Kaolin in the recent past has been of interest in clinical studies due to its role in achieving haemostasis in Oculoplastic Surgery as a local application<sup>456</sup> or intra-abdominal surgery with Kaolin impregnated gauze as a leave-in substance for rapid haemorrhage control in critically injured patients in combat<sup>457</sup>.

Kaolin has the universal property of causing a foreign body reaction and inducing an inflammatory response that induces adhesion formation<sup>458,459</sup>. The injury is similar to the mesothelial injury in abdominal adhesion by foreign body reaction and setting up a wound healing process resulting in fibrosis/adhesion as seen in pulmonary fibrosis<sup>448</sup>.

The pathology thus generated could replicate the human condition of tissue handling, glove powder, mechanical injury due to clamps and electrocautery. A rat model is relatively easy to use and replicate in terms of the experiment and also the ratio of the peritoneal surface area relative to the body weight and height is comparable to human<sup>368</sup>. The volume of adhesion inducing agent also matters when we test an anti-adhesive substance, hence refinement of 4 mL to 2 mL is significant in terms of animal discomfort post-surgery. The surface area in the rat abdomen is high but the volume of chemical used to induce injury has to be titrated sufficient enough to cause injury and provide space for the anti-adhesive agent. The dosage of Kaolin that’s ideal in both the laparotomy with abrasion alone and abrasion with enterotomy model was 0.005g/ml and this produces consistent adhesion without being harmful to the rat. Interestingly as expected there was higher grade of adhesion seen with the

enterotomy model in compared to the laparotomy group, but the rats were able to tolerate the insult and recovered without any morbidity or weight loss.

One of the limitations in this model is the anti-coagulant property of Kaolin which may inhibit adhesion formation<sup>457</sup>. In spite of which, the overall ability of its property to induce chemical injury has resulted in a controlled amount of adhesion formation using a low dosage.

In conclusion, our rat model for abdominal adhesion prevention experiments using Kaolin silicate at a dosage of 0.005g/mL is safe and efficacious.



### **7.1.2 Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a Rat Model**

Conducted in the Department of Otolaryngology – Head and Neck Surgery

The University of Adelaide, Adelaide, Australia,

Financial assistance provided by The University of Adelaide and The Hospital Research

Foundation under Australian Government Research Training Program Scholarship

## RESEARCH ARTICLE

## Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a rat model

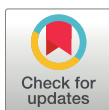
Rajan Sundaresan VEDIAPPAN<sup>1</sup>✉, Catherine BENNETT<sup>1</sup>✉, Clare COOKSLEY<sup>1</sup>✉, John FINNIE<sup>2</sup>✉, Markus TROCHSLER<sup>3</sup>✉, Ryan D. QUARRINGTON<sup>4</sup>✉, Claire F. JONES<sup>4,5</sup>✉, Ahmed BASSIOUNI<sup>1</sup>✉, Stephen MORATTI<sup>6</sup>✉, Alkis J. PSALTIS<sup>1</sup>✉, Guy MADDERN<sup>3</sup>✉, Sarah VREUGDE<sup>1</sup>✉, P. J. WORMALD<sup>1</sup>✉\*

**1** Department of Surgery—Otolaryngology Head and Neck Surgery, The University of Adelaide, Adelaide, Australia, **2** SA Pathology and Adelaide Medical School, The University of Adelaide, Adelaide, Australia, **3** Department of Surgery, The University of Adelaide, Adelaide, Australia, **4** Adelaide Spinal Research Group, Centre for Orthopaedic and Trauma Research, Adelaide Medical School, University of Adelaide, Adelaide, Australia, **5** School of Mechanical Engineering, University of Adelaide, Adelaide, Australia, **6** Department of Chemistry, Otago University, Dunedin, New Zealand

✉ These authors contributed equally to this work.

‡ These authors also contributed equally to this work.

\* [peterj.wormald@adelaide.edu.au](mailto:peterj.wormald@adelaide.edu.au)



## OPEN ACCESS

**Citation:** VEDIAPPAN RS, BENNETT C, COOKSLEY C, FINNIE J, TROCHSLER M, QUARRINGTON RD, et al. (2021) Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a rat model. *PLoS ONE* 16(1): e0244503. <https://doi.org/10.1371/journal.pone.0244503>

**Editor:** Aleksandar R. Zivkovic, Heidelberg University Hospital, GERMANY

**Received:** May 15, 2020

**Accepted:** December 10, 2020

**Published:** January 14, 2021

**Peer Review History:** PLOS recognizes the benefits of transparency in the peer review process; therefore, we enable the publication of all of the content of peer review and author responses alongside final, published articles. The editorial history of this article is available here: <https://doi.org/10.1371/journal.pone.0244503>

**Copyright:** © 2021 VEDIAPPAN et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Data Availability Statement:** All relevant data are within the manuscript and its [Supporting Information](#) files.

## Abstract

## Introduction

Adhesions are often considered to be an inevitable consequence of abdominal and pelvic surgery, jeopardizing the medium and long-term success of these procedures. Numerous strategies have been tested to reduce adhesion formation, however, to date, no surgical or medical therapeutic approaches have been successful in its prevention. This study demonstrates the safety and efficacy of Chitogel with Deferiprone and/or antibacterial Gallium Protoporphyrin in different concentrations in preventing adhesion formation after abdominal surgery.

## Materials and methods

112 adult (8–10 week old) male Wistar albino rats were subjected to midline laparotomy and caecal abrasion, with 48 rats having an additional enterotomy and suturing. Kaolin (0.005g/ml) was applied to further accelerate adhesion formation. The abrasion model rats were randomized to receive saline, Chitogel, or Chitogel plus Deferiprone (5, 10 or 20 mM), together with Gallium Protoporphyrin (250µg/mL). The abrasion with enterotomy rats were randomized to receive saline, Chitogel or Chitogel with Deferiprone (1 or 5 mM). At day 21, rats were euthanised, and adhesions graded macroscopically and microscopically; the tensile strength of the repaired caecum was determined by an investigator blinded to the treatment groups.

## Results

Chitogel with Deferiprone 5 mM significantly reduced adhesion formation ( $p < 0.01$ ) when pathologically assessed in a rat abrasion model. Chitogel with Deferiprone 5 mM and 1 mM

## Statement of Authorship

|                     |                                                                                                                                                                                                                                                |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title of Paper      | Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a Rat Model                                                                                                                  |
| Publication Status  | <input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication<br><input type="checkbox"/> Submitted for Publication<br><input type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Submitted for                                                                                                                                                                                                                                  |

### Principal Author

|                                      |                                                                                                                                                                                                                                                                                                |      |         |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|---------|
| Name of Principal Author (Candidate) | Vediappan, Rajan Sundaresan                                                                                                                                                                                                                                                                    |      |         |
| Contribution to the Paper            | Conducted the Study, collected data, analysis, interpretation, drafting manuscript, editing and submission of manuscript.                                                                                                                                                                      |      |         |
| Overall percentage (%)               | 70%                                                                                                                                                                                                                                                                                            |      |         |
| Certification:                       | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |      |         |
| Signature                            |                                                                                                                                                                                                                                                                                                | Date | 15.0520 |

### Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above).
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

|                           |                                                            |      |         |
|---------------------------|------------------------------------------------------------|------|---------|
| Name of the Author        | Bennett, Catherine                                         |      |         |
| Contribution to the Paper | Conduct of study, Collection of data & drafting paper -10% |      |         |
| Signature                 |                                                            | Date | 15.0520 |
| Name of the Author        | Cooksley, Clare                                            |      |         |

## Modifying wound healing and PO outcome

|                           |                                                                                                |      |          |
|---------------------------|------------------------------------------------------------------------------------------------|------|----------|
| Contribution to the Paper | Conduct of study, Collection of data & drafting paper -1%                                      |      |          |
| Signature                 |                                                                                                | Date | 15.0520  |
| Name of the Author        | Finnie, John                                                                                   |      |          |
| Contribution to the Paper | Conduct of study, Collection of data & drafting paper -2%                                      |      |          |
| Signature                 |                                                                                                | Date | 15.0520  |
| Name of the Author        | Trochsler, Markus                                                                              |      |          |
| Contribution to the Paper | Conduct of study, Collection of data & drafting paper 1%                                       |      |          |
| Signature                 |                                                                                                | Date | 15.0520  |
| Name of the Author        | Quarrington, Ryan D                                                                            |      |          |
| Contribution to the Paper | Conduct of study, Collection of data, Analysis and Interpretation of data & drafting paper -2% |      |          |
| Signature                 |                                                                                                | Date | 15.05.20 |
| Name of the Author        | Jones, Claire F                                                                                |      |          |
| Contribution to the Paper | Conduct of study, Collection of data, Analysis and Interpretation of data & drafting paper -1% |      |          |
| Signature                 |                                                                                                | Date | 15.05.20 |
| Name of the Author        | Bassiouni, Ahmed                                                                               |      |          |
| Contribution to the Paper | Analysis and Interpretation of data & drafting paper -1%                                       |      |          |
| Signature                 |                                                                                                | Date | 15.05.20 |
| Name of the Author        | Moratti, Stephen                                                                               |      |          |

|                           |                                                                                                                                                                |      |          |
|---------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------|----------|
| Contribution to the Paper | Conduct of study, Intellectual and critical input towards final draft of paper -1%                                                                             |      |          |
| Signature                 |                                                                                                                                                                | Date | 15.05.20 |
| Name of the Author        | Psaltis, Alkis J                                                                                                                                               |      |          |
| Contribution to the Paper | Interpretation of Data, Critical input and approval for submission -1%                                                                                         |      |          |
| Signature                 |                                                                                                                                                                | Date | 15.05.20 |
| Name of the Author        | Vreugde, Sarah                                                                                                                                                 |      |          |
| Contribution to the Paper | Design, Interpretation of results, revising it critically for important intellectual content and approval for submission – 5%                                  |      |          |
| Signature                 |                                                                                                                                                                | Date | 15.05.20 |
| Name of the Author        | Wormald, Peter John                                                                                                                                            |      |          |
| Contribution to the Paper | Conceptualization, Design, Conduct, Interpretation of results, revising it critically for important intellectual content and final approval for submission -5% |      |          |
| Signature                 |                                                                                                                                                                | Date | 15.05.20 |

## **Prevention of adhesions post-abdominal surgery: Assessing the safety and efficacy of Chitogel with Deferiprone in a Rat Model**

Rajan Sundaresan VEDIAPPAN<sup>1¶</sup>, Catherine Bennett<sup>1¶</sup>, Clare Cooksley<sup>1¶</sup>, John Finnie<sup>2¶</sup>, Markus Trochsler<sup>3¶</sup>, Ryan D Quarrington<sup>4&</sup>, Claire F Jones<sup>4,5&</sup>, Ahmed Bassiouni<sup>1¶</sup>, Stephen Moratti<sup>6¶</sup>, Alkis J Psaltis<sup>1¶</sup>, Guy Maddern<sup>3¶</sup>, Sarah Vreugde<sup>1¶</sup>, Wormald PJ<sup>1¶\*</sup>

<sup>1</sup>Department of Surgery - Otolaryngology Head and Neck Surgery, The University of Adelaide, Australia

<sup>2</sup>SA Pathology and Adelaide Medical School, The University of Adelaide, Adelaide, Australia

<sup>3</sup>Department of Surgery, The University of Adelaide, Australia

<sup>4</sup>Adelaide Spinal Research Group, Centre for Orthopaedic and Trauma Research, Adelaide Medical School, University of Adelaide, Adelaide, Australia

<sup>5</sup>School of Mechanical Engineering, University of Adelaide, Adelaide, Australia

<sup>6</sup>Department of Chemistry, Otago University, Dunedin, New Zealand

### **\* Corresponding Author:**

Professor Peter John Wormald

The University of Adelaide

Department of Surgery - Otolaryngology, Head & Neck Surgery

The Queen Elizabeth Hospital

28, Woodville Road, Woodville south-5011

Adelaide, South Australia

Contact: peterj.wormald@adelaide.edu.au

Ph: (+61 8) 8222 7158 Fax: (+61 8) 8222 7419

**Author Contributions:** <sup>¶</sup>RSV, CB, CC, MT, JF, <sup>&</sup>RQ, <sup>&</sup>CJ & AB involved in the conduct of the study, collection of data and interpretation of the result with drafting and editing the manuscript <sup>¶</sup>SM, AJP, GM, SV & PJW were involved in designing the study, supervision, Interpretation of data, drafting and editing the manuscript and final permission for submission.

**Conflict of Interest:** PJW and SV are inventors on intellectual property concerning Gallium Protoporphyrin and Deferiprone for use in the prevention of scarring; PJW and SM are shareholders in Chitogel. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

**Funding:** This project is supported by an NHMRC Development grant APP1171756 awarded to SM, SV, GM and MT.

**Acknowledgements:** RSV was supported by The Hospital Research Foundation and The University of Adelaide under Australian Government Research Training Program Scholarship; the authors would also like to appreciate the contribution of The Queen Elizabeth Hospital Animal experimental suite and the Basil Hetzel Institute for their support during the study.

**Abstract:**

**Introduction:** Adhesions are often considered to be an inevitable consequence of abdominal and pelvic surgery, jeopardizing the medium and long-term success of these procedures. Numerous strategies have been tested to reduce adhesion formation, however, to date, no surgical or medical therapeutic approaches have been successful in its prevention. This study demonstrates the safety and efficacy of Chitogel with Deferiprone and/or antibacterial Gallium Protoporphyrin in different concentrations in preventing adhesion formation after abdominal surgery.

**Materials and Methods:** 112 adult (8-10 week old) male Wistar albino rats were subjected to midline laparotomy and caecal abrasion, with 48 rats having an additional enterotomy and suturing. Kaolin (0.005g/ml) was applied to further accelerate adhesion formation. The abrasion model rats were randomized to receive saline, Chitogel, or Chitogel plus Deferiprone (5, 10 or 20 mM), together with Gallium Protoporphyrin (250µg/mL). The abrasion with enterotomy rats were randomised to receive saline, Chitogel or Chitogel with Deferiprone (1 or 5 mM). At day 21, rats were euthanised, and adhesions graded macroscopically and microscopically; the tensile strength of the repaired caecum was determined by an investigator blinded to the treatment groups.

**Results:** Chitogel with Deferiprone 5 mM significantly reduced adhesion formation ( $p<0.01$ ) when pathologically assessed in a rat abrasion model. Chitogel with Deferiprone 5 mM and 1 mM also significantly reduced adhesions ( $p<0.05$ ) after abrasion with enterotomy. Deferiprone 1mM treatment did not weaken the enterotomy site with treated sites having significantly better tensile strength compared to control saline treated enterotomy rats.



**Conclusions:** Chitogel with Deferiprone 1 mM constitutes an effective preventative anti-adhesion barrier after abdominal surgery in a rat model. Moreover, this therapeutic combination of agents is safe and does not weaken the healing of the sutured enterotomy site.

Key words: Chitogel, Deferiprone, Abdominal adhesion, Animal model, fibrosis

### **Introduction:**

Seven million open abdominal surgeries occur each year in the US and Europe <sup>446</sup>, costing the health care system \$USD 2.3 billion annually <sup>302</sup>. However, postsurgical adhesions are an almost inevitable consequence of abdominal surgery and are the largest single cause of intestinal obstruction<sup>288</sup>. Occurrence of adhesions after upper and lower abdominal surgery ranges from 67-93% <sup>24,273</sup>. The mortality rate due to postsurgical adhesions can be high, especially among the elderly <sup>460</sup>, and these complications can cause chronic pain and female infertility <sup>274,447</sup>. Prevention of adhesions aims to reduce inflammation and infection, which are the main triggers of their formation. After surgery, inflammation results in extravasation of a fibrinogen-rich fluid, the resulting fibrin clot promoting adhesion formation, a process accentuated by microbial contamination from leaked intestinal contents.

Numerous strategies have been devised to prevent peritoneal adhesions, such as hydro flotation, barrier agents such as anti-adherence hyaluronic acid/carboxymethylcellulose, regenerated and expanded oxidised cellulose 0.5% in ferric hyaluronate and chlorine dioxide <sup>273</sup>. However, none of these strategies have been widely adopted due to poor efficacy or risk of adverse events <sup>305</sup>. An ideal barrier agent should be a biocompatible substance that is sufficiently flexible to conform to the abdominal cavity and able to be used during laparotomy or laparoscopy. It should also be able to adhere to the peritoneal surface and

remain in-situ for 5 to 7 days after the surgery. Moreover, it should prevent thrombin formation and hydrolyse, without leaving degraded residue that is pro-inflammatory in nature.

Chitogel® has been identified as an ideal candidate for this role. It is a dissolvable gel that can carry Deferiprone (Def), an iron chelator, and Gallium-Protoporphyrin<sup>89</sup> an anti-bacterial haem analogue. Chitogel has been used extensively in the nasal cavity and sinuses as a haemostatic and adhesion prevention agent with considerable success. It has good haemostatic<sup>44,49</sup> and anti-adhesive properties<sup>44,80,252</sup>, and an anti-microbial action<sup>80</sup>. Chitogel is biocompatible, non-toxic<sup>42,444</sup>, an excellent drug delivery device, and is currently a Food and Drug Administration (FDA) approved postoperative dressing in sinuses post-surgery. Previous *in vivo* studies conducted in small and large animal models of abdominal surgery support Chitogel's anti-adhesive properties within the abdominal cavity<sup>332,362</sup>. Def is an FDA-approved drug for the treatment of iron-overload conditions such as Thalassemia Major, which has also been shown to reduce reactive oxygen species (ROS), an important contributor to the inflammatory process in wound healing. *In vitro* Def has also been shown to reduce the migration and proliferation of fibroblasts in a time and dose-dependent manner<sup>85</sup>. Importantly, Def is released from Chitogel within 48 to 72 hours, a critical timeframe for the prevention of adhesion development<sup>365</sup>.

GaPP has a similar structure to haem, with Gallium complexed in its center rather than iron. Bacteria require iron for their metabolism and actively absorb GaPP. When used in combination with Def, Def-GaPP has demonstrated potent synergistic anti-microbial effects, killing both Gram-positive and Gram-negative bacteria, including Multi Drug Resistant (MDR) bacteria<sup>461</sup>. GaPP is released from Chitogel for up to 460 hours *in vitro* and *in vivo*<sup>365</sup>, making it available to bacteria long term.

This study sought to determine the lowest therapeutically relevant dose of Def required to effectively reduce adhesion formation after abdominal surgery.

### **Materials & Methods:**

The University of Adelaide and Central Adelaide Local Health Network/SA Pathology Animal Ethics Committees (AEC) approved the study to be conducted at The Queen Elizabeth Hospital Experimental Surgical Suite (The University of Adelaide AEC M-2017-061 and CALHN/SA Pathology AEC 25-17) and the AHMS Biomechanics Laboratory.

#### **Animals:**

Male Wistar albino rats were purchased from Laboratory Animal Services

Medical School (The University of Adelaide, SA, Australia), 8 to 10 weeks old, with an average weight between 350 and 500 grams. Rats were housed 1 week prior to surgery under standard laboratory conditions (temperature  $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , humidity  $55\% \pm 10\%$ , 12: 12-hour light-dark-cycle). Rats were housed in groups of 3 per cage and food and water were provided in a standard manner.

#### **Materials**

Kaolin (Aluminium silicate Hydroxide,  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ ) was purchased from Sigma-Aldrich, St. Louis, Missouri, United States (k7375-500G, Lot#SLN7548V).

#### **Chitogel**

The Chitogel is made up of a combination of three components: 1% succinyl chitosan, 0.3% phosphate buffer +/- 40% glycerol and 3% dextran aldehyde (Chitogel, Wellington, NZ),<sup>462</sup>

MXR303 . The components are manufactured and sterilized by Chitogel. All stocks were stored at room temperature.

#### Deferiprone and Gallium Protoporphyrin

Deferiprone (3-hydroxy-1,2-dimethylpyridin-4(1H)-one) (Sigma-Aldrich, St Louis, USA) (Lot # STBG8424) and Gallium Protoporphyrin IX (Ga-PP IX) (Frontier Scientific, Logan, USA) ( Lot # JB18-12460) were stored at room temperature.

#### Preparation of Chitogel

Dextran aldehyde (0.3 g) was dissolved in 10 mL of phosphate buffer +/- 40% glycerol then mixed with 10 mL 1% succinyl chitosan.

#### Preparation of Chitogel-Deferiprone-Gallium Protoporphyrin

Deferiprone (80 mM, 40 mM, 20 mM or 4mM) and Gallium Protoporphyrin (1 mg/mL) were dissolved in 5 mL phosphate buffer (+/- 40% glycerol) under sterile conditions. For Def/GaPP combination gel, 5 mL of each were added to dissolve dextran aldehyde prior to mixing with 10 mL of 1% succinyl chitosan. For Def gel, 5 mL Def solution plus 5 mL buffer were added to dissolve dextran aldehyde prior to mixing with 10 mL of 1% succinyl chitosan.

#### Surgical procedure:

Surgical procedures were performed by the same surgeons (RSV, CB) and a maximum group size of five animals per day was used to ensure close monitoring during the immediate post-operative period. Anaesthesia was achieved using a sealed chamber to deliver 2-3% Isoflurane, after which the animal was positioned supine for surgery and anaesthesia maintained with isoflurane over an open mask. Analgesia was

provided preoperatively by subcutaneous injection of Buprenorphine (0.05 mg/kg) and post-operative 8 hourly for 48 hours. The surgery was conducted in aseptic manner and a prophylactic dosage of broad-spectrum antibiotic in the form of Amoxycillin Clavulanic acid 5 mg/kg (Clavulox\* Zoetis Australia, Rhodes, NSW, Australia) was also administered via subcutaneous injection.

Rats underwent a laparotomy and a colon abrasion<sup>332</sup> or a colon abrasion with enterotomy<sup>463</sup>. Briefly, the abdomen was shaved and prepared with alcohol. After drying, a 3 cm laparotomy (Fig 1a) was performed to gain access to the abdominal cavity. The caecum was delivered and kept moist with saline-soaked gauze whilst a dry gauze was used to rub the caecum repeatedly until sub-serosal bleeding occurred over an area of 1 cm<sup>2</sup> (Fig 1 b & c). 2 ml 0.005 g/mL Kaolin in saline was instilled over the abrasion<sup>463</sup>. The caecum was then returned to the abdomen and the abdominal wall closed in layers with a 3-0 Polyglactin suture. Prior to the placement of the final abdominal closure suture, rats were randomized to receive the following treatments into the abdominal cavity:

- (1) 4 mL normal saline, n=12
- (2) 4 mL Chitogel, n=12
- (3) 4 mL Chitogel + Def 20 mM + GaPP 250 µg/mL, n=12
- (4) 4 mL Chitogel + Def 10 mM + GaPP 250 µg/mL, n=12
- (5) 4 ml Chitogel + Def 5 mM + GaPP 250 µg/mL, n=12

In the Caecal abrasion + enterotomy group rats, colon abrasion (dry rubbing of the caecum wall over an area of 1 cm<sup>2</sup> with gauze until bleeding occurs) and a full thickness enterotomy of the caecum over a length of 10 mm at an adjacent site of Caecum was performed. The enterotomy was then closed with 4-0 PDS suture (resorbable, monofilament) ( Fig 1d) and

the repair leak tested with a simple pressure test. 2 ml 0.005 g/mL Kaolin in saline was instilled over the abrasion and sutured site, followed by application of 4 ml of the test treatments without glycerol into the abdomen by randomization before closure of the abdominal wall as follows:

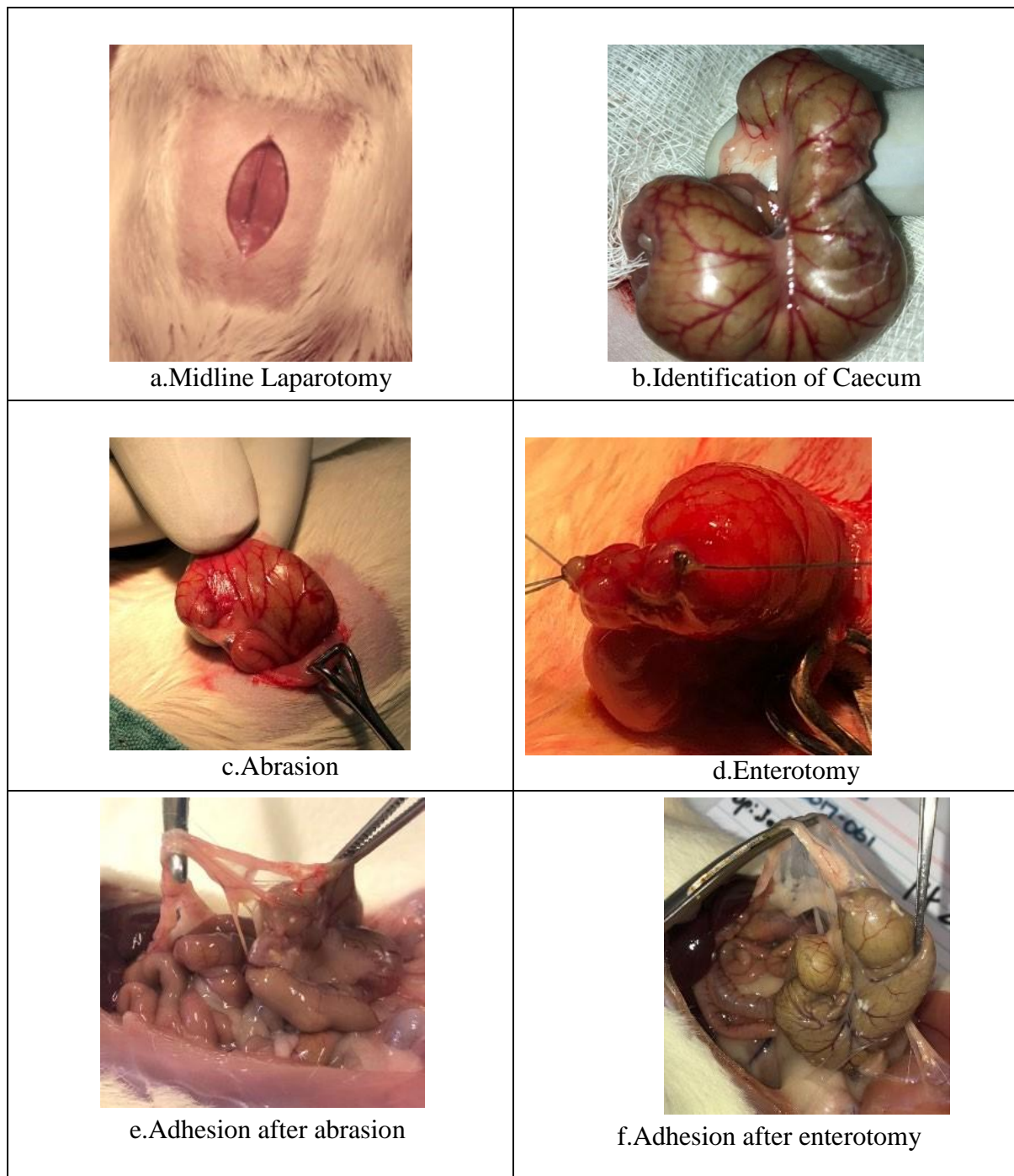
- (1) 4 mL normal saline, n=12
- (2) 4 mL Chitogel, n=12
- (3) 4 mL Chitogel + Def 5 mM n=12
- (4) 4 mL Chitogel + Def 1 mM n=12

The operation was limited to <15-20 mins each rat so as to avoid air drying of the organs.

#### Postoperative monitoring

Post-surgery, the animals were housed individually in separate cages at a constant room temperature with a 12 h light and dark cycle. In the immediate post-operative period animals were given Lectade Oral Rehydration Therapy (Lectade, Jurox Pty Limited, Australia) until they were able to eat standard rodent food and drink water that were provided *ad libitum*. Animals were monitored every 8-hours for the first 48 hours post-surgery. Their weight, behaviour, physical well-being, and appearance were documented using the Clinical Record Sheet, as approved by AEC. Adequate pain relief was maintained until 72 h post-surgery, and distress scores higher than 6 or weight loss greater than 15% required that animals be euthanised.

Outcome measures:



**Figure1:** a. Incision over the Rat abdominal wall after preparation, (orange arrow)

b. Identification of Caecum, c. Abrasion over the caecum with gauze till bleeding spots appear, d. Enterotomy sutured, e. Adhesion induced by Kaolin at dosage of 0.005g/ml at day 21, f. Adhesion over the enterotomy site at day 21

The animals were humanely killed using a CO<sub>2</sub> gas inhalation chamber after 21 days post-operative observation. Post-mortem laparotomy was performed to assess adhesion formation based on the presence and severity of adhesions using a previously validated adhesion scoring system (Table 1)<sup>332</sup>. The score takes into account the number, strength and distribution of adhesions formed. Pictures were taken with an iPhone 8 12mp *f*/1.8 aperture camera (Fig 1 e & f) and a macroscopic grade was assigned to each rat by an abdominal surgeon who was blinded to treatment. The intra-abdominal cavity was examined for any residual gel and contents were examined for any gross changes.

Table 1. Adhesion Scoring Scheme

| Adhesion Scoring Scheme | Score Description                                      |
|-------------------------|--------------------------------------------------------|
| 0                       | No adhesions                                           |
| 1                       | Thin adhesion strands                                  |
| 2                       | Multiple thin adhesions                                |
| 3                       | Thick adhesion with focal attachment                   |
| 4                       | Thick adhesion with more broad-based planar attachment |
| 5                       | Massive adhesions or more than one planar adhesion     |

#### Histology:

The caecum with adhesion(s) was collected and the tissue between the caecal adventitia and adherent adjacent intestinal serosal surfaces, and between the adventitial aspect of the caecum and the parietal peritoneum of the abdominal wall, were collected and immersion-fixed in 10% neutral buffered formalin. These tissues were then paraffin-embedded, cut at 6 µm, and



stained with haematoxylin and eosin (H&E). Duplicate sections were also stained by the Masson's trichrome technique to demonstrate collagen deposition in fibrous adhesions. The slides were examined, and scored, independently by two observers, blinded as to the treatment groups.

In order to assess the nature of the intestinal adhesions produced by our experimental paradigms, we attempted to grade these adhesions with respect to the stage of foreign body inflammatory reaction and degree of fibrosis.

Since there was some variability in the stage of these pathological processes between different intestinal sites in a given animal, the adhesions were initially scanned at low magnification (x4) and 3 sites selected for further analysis (at x20 magnification), these being areas of adhesions most representative of the overall pathological reaction in each case (Fig 2).

In routine H&E - stained sections, the 3 sites selected were scored for inflammation and wound healing according to an internally validated scoring system (Table 2):

Table 2, Grade of Inflammation and cellular proliferation in Adhesion (H&E)

| Grade    | Description                                                                                                                                                                         |
|----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Grade 1+ | predominantly epithelioid macrophage infiltration, with phagocytosed adhesion-inciting material, and fewer multinucleated giant cells, lymphocytes and plasma cells                 |
| Grade 2+ | fibrovascular granulation tissue formation, with fibroblastic proliferation and supporting microvascular angiogenesis, and a relatively small quantity of loosely arranged collagen |
| Grade 3+ | more mature fibroplasia with abundant collagen deposition                                                                                                                           |

These 3 sites were also evaluated in sections stained by the Masson's trichrome technique for quantity and quality of collagen deposition (Fig 3), and the Grades are shown in Table 3:

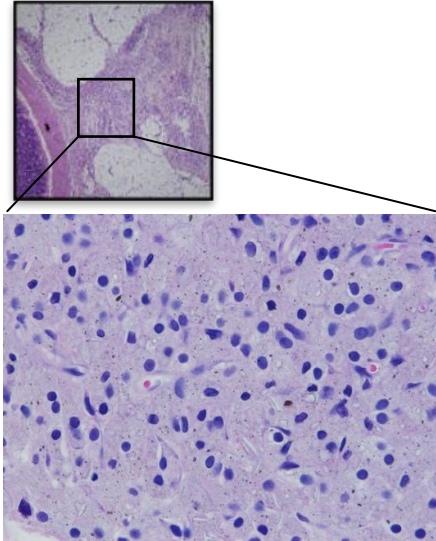
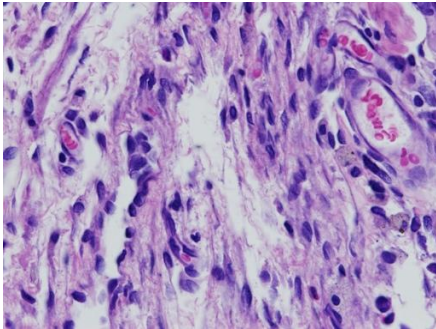
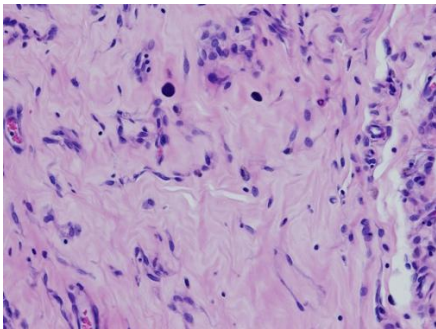
|    |                                                                                                                                                                                                                                                     |                                                                                      |
|----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| 1+ | <ul style="list-style-type: none"> <li>• predominantly epithelioid macrophage infiltration,</li> <li>• phagocytosed adhesion-inciting material</li> <li>• fewer multi-nucleated giant cells, lymphocytes and plasma cells</li> </ul>                |    |
| 2+ | <ul style="list-style-type: none"> <li>• fibrovascular granulation tissue formation</li> <li>• fibroblastic proliferation, supporting microvascular angiogenesis</li> <li>• and a relatively small quantity of loosely arranged collagen</li> </ul> |  |
| 3+ | <ul style="list-style-type: none"> <li>• more mature fibroplasia</li> <li>• abundant collagen deposition</li> </ul>                                                                                                                                 |  |

Figure 2: H & E grading of Caecal scar tissue 20X

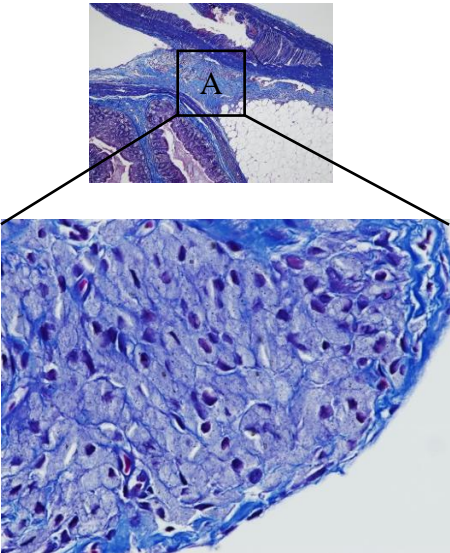
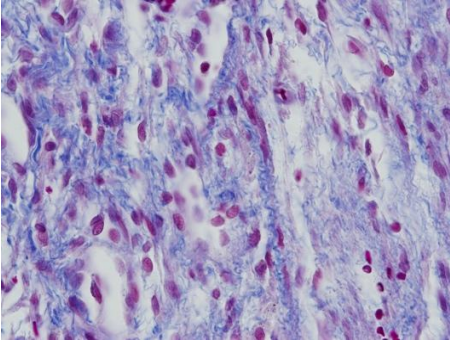
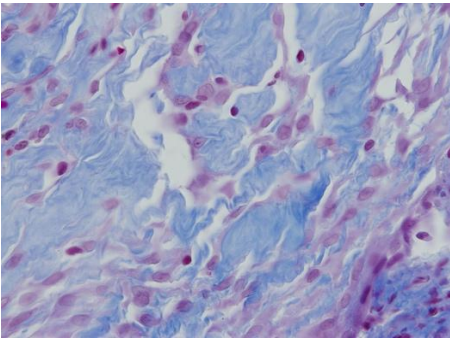
|           |                                                                                                                                                                                    |                                                                                      |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <p>1+</p> | <ul style="list-style-type: none"> <li>• minimal and loosely arranged collagen fibrils</li> <li>• numerous fibroblastic nuclei</li> </ul>                                          |    |
| <p>2+</p> | <ul style="list-style-type: none"> <li>• more abundant</li> <li>• compactly arranged collagen deposition</li> </ul>                                                                |   |
| <p>3+</p> | <ul style="list-style-type: none"> <li>• mature collagenous connective tissue with compact,</li> <li>• dark blue-staining collagen</li> <li>• fewer fibroblastic nuclei</li> </ul> |  |

Table 3, Grade of Fibroblastic activity in the Adhesion (Masson's Trichrome)

| Grade    | Description                                                                                       |
|----------|---------------------------------------------------------------------------------------------------|
| Grade1+  | minimal and loosely arranged collagen fibrils with numerous fibroblastic nuclei                   |
| Grade 2+ | more abundant and compactly arranged collagen deposition                                          |
| Grade 3+ | mature collagenous connective tissue with compact collagen and markedly fewer fibroblastic nuclei |

#### Tensile strength testing:

After separation for histology, the caecal tissue was laid open and cut into a rectangular specimen (nominally 40 mm long and 9 mm wide) centred about the suture site (for the treatment groups). The ends (5 mm) of each specimen were attached to custom plastic gripping tabs (20×20 mm) using cyanoacrylate adhesive (Loctite 401, Henkel, Düsseldorf, Germany), giving a gauge length of approximately 30 mm (Fig 4 a). These gripping tabs assisted with fixing the specimen into an electromechanical tensile testing machine (5543, Instron, High Wycombe, UK) via pneumatic grips (2712-019, Instron, High Wycombe, UK; 5 bar compressed air pressure). Specimens were consistently placed in the grips with thicker colonic wall superiorly. Prior to testing, a tensile pre-load of 0.01 N was applied; the specimen width above, below, and at the suture site (middle for naïve tissue group), and specimen gauge length, were measured using Vernier callipers (make, model etc). Tensile loading was applied at 0.1 mm/s until complete failure occurred (Fig. 5 A). Loads and displacements were recorded at 100 Hz using a uniaxial load cell (range  $\pm 10$  N, Instron, High Wycombe, UK) and linear variable differential transducer (position accuracy  $\pm 0.02$  mm), respectively. All tests were video recorded in high-definition using a mobile-phone camera for qualitative analysis of the failure region.

Custom MATLAB code (R2015a, MathWorks, Massachusetts, USA) was developed to filter the load and displacement data using a second-order, two-way Butterworth low-pass filter with a cut-off frequency of 10 Hz, and load-displacement plots were generated. The peak load and extension (displacement) at peak load were calculated. Stiffness (N/mm) was determined from the linear region, the bounds of which were determined by a single operator, and a linear regression line was fitted to the data points within this region to determine the slope.

#### Statistical Analysis:

Statistics of adhesion grades and histology were performed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria) through the Jupyter notebook interface. The R package "MASS"<sup>442</sup> was used for ordinal regression. The "polr" function from MASS was used to fit a proportional odds logistic regression model for the ordinal outcome variable (the adhesion score as scored by the primary surgeons). A Likelihood ratio test (using the R function "anova") was used to compare the model with a null ordinal regression model. The means of the ordinal response (interpreted as a numeric value from 1 to the number of classes) were calculated and post-hoc pairwise contrasts for each pair of levels of the treatment variable were compared using the "emmeans" package.<sup>442</sup> Statistical significance was taken at the traditional  $< 0.05$  level.

Statistical analyses for the mechanical testing were performed using SPSS v22 (IBM, Illinois, USA). Three linear regression models were developed to identify if treatment (naive, control, gel alone, Chitogel with 1 mmol or 5 mmol of Def) was significantly associated with the following outcome measures: 1) peak load, 2) extension at peak load, and 3) linear region stiffness. Each model was developed as follows: Firstly, Shapiro-Wilk and Levene tests were

performed to assess normality and homogeneity of variance of the dependent variables, respectively. If required, statistically significant outliers were removed to meet these criteria. The effect of treatment was assessed in all models, and this effect was adjusted for the geometric measurements taken of the specimens when under 0.01 N preload: thick-end width, middle (or suture site) width, thin-end width, and length. Each model was refined using a manual backward stepwise approach until only significant predictors remained ( $\alpha=0.05$ ). Bonferroni-adjusted post-hoc comparisons were used to determine differences between treatment group.

## **Results:**

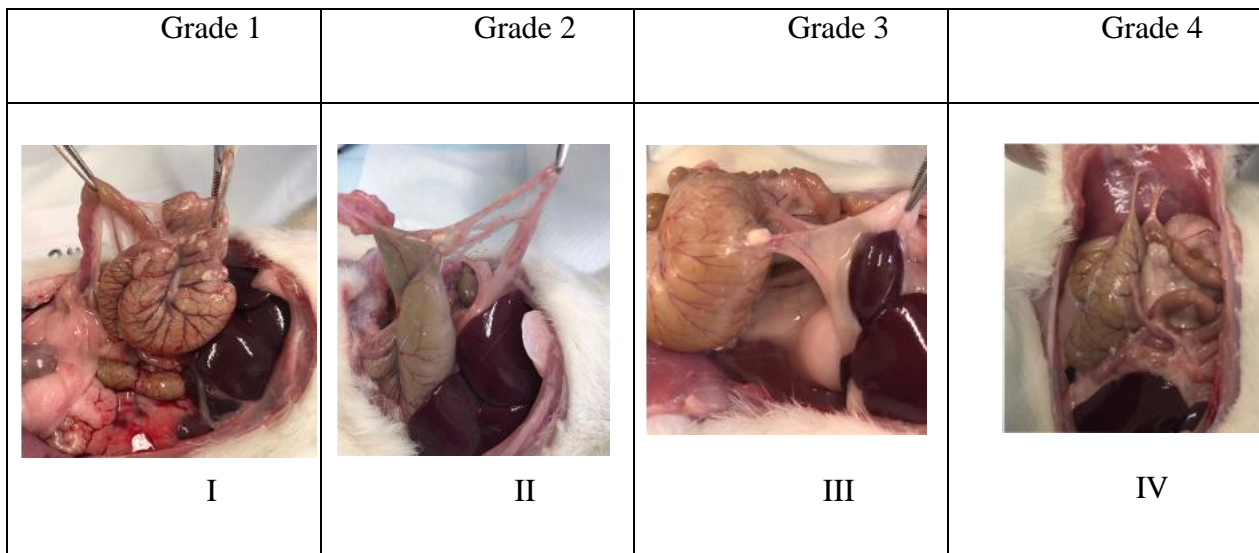
### Macroscopic Adhesion Scores

One hundred and eight rats underwent either colon abrasion (n=60) or colon abrasion with enterotomy (n=48). Post-operative follow-up was uneventful for all rats with no major complications up to day 21 after surgery. All 108 rats were recovered and at day 21 humanely killed and observed for adhesions. All major organs were un-affected, and the abdominal cavity was free of Kaolin or any residual products of Chitogel.

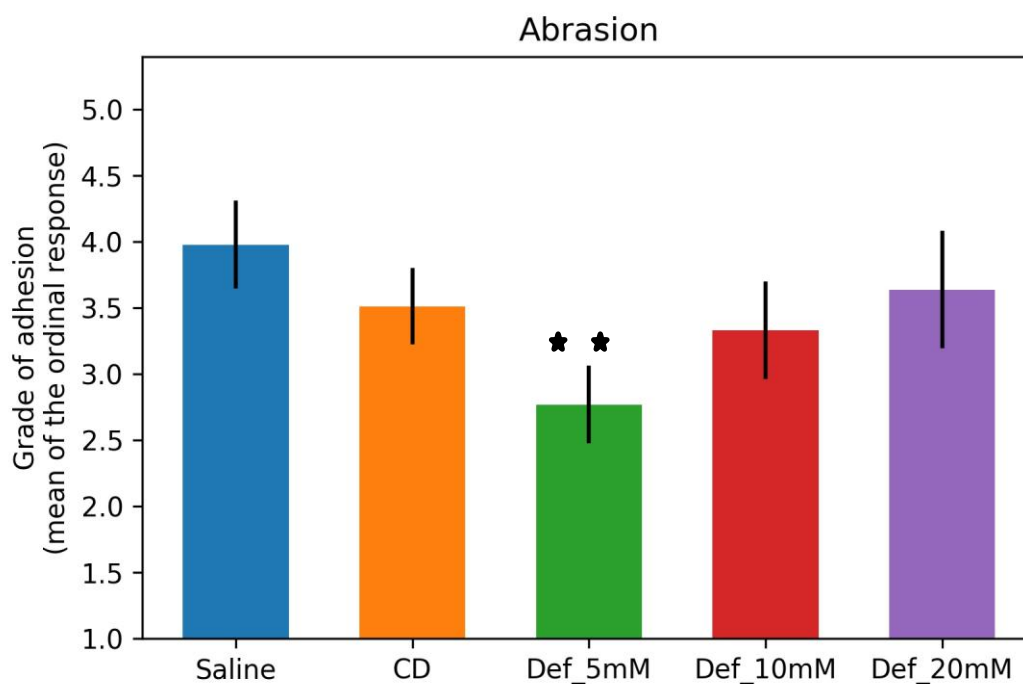
### Abrasion Model

The mean adhesion score in control rats treated with only saline was 3.98 (CI 3.33, 4.63), and there were thick adhesions present over the site of abrasion in most of the rats (Fig. 4A, IV). Some were vascularized and had planar attachments between the abdominal wall and the site of injury (Fig. 4A III). The rats treated with Chitogel and Deferiprone 5 mM showed a significant reduction of the intra-abdominal adhesion scores macroscopically with a mean adhesion score of 2.77 (CI 2.19, 3.4) ( $p<0.01$ ) (Fig 4 B). Some of these rats had a few very

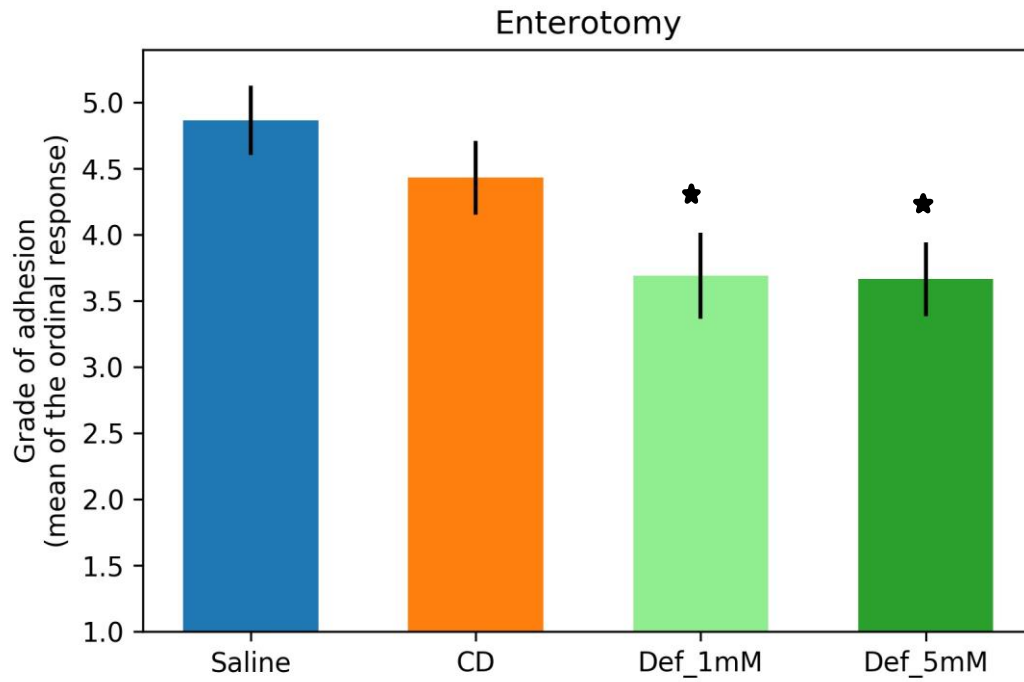
thin adhesion strands and in some there was more than one adhesion strand (Fig. 5, II). Rats treated with Chitogel alone had a mean adhesion score of 3.51 (CI 2.95, 4.08) and higher dosages of Def 10 mM and Def 20 mM had similar mean adhesion scores of 3.33 (CI 2.6, 4.06) & 3.64 (CI 2.77, 4.51) respectively.



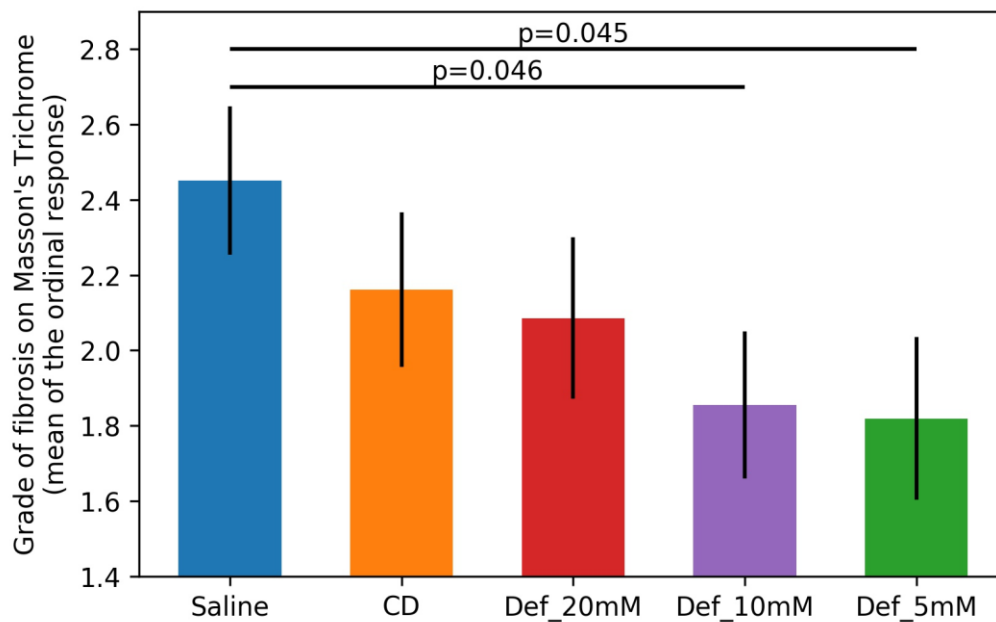
**Figure 4A; Photographs of rat abdomen at end point depicting different grades of adhesion**



**Figure 4 B; Blinded grading of Adhesion in an abrasion model based on Table 2**



**Figure 4 C; Blinded grading of Abdominal adhesion based on table 2**



**Figure 4 D Blinded grading of Grade of Fibrosis on Masson's Trichrome staining**



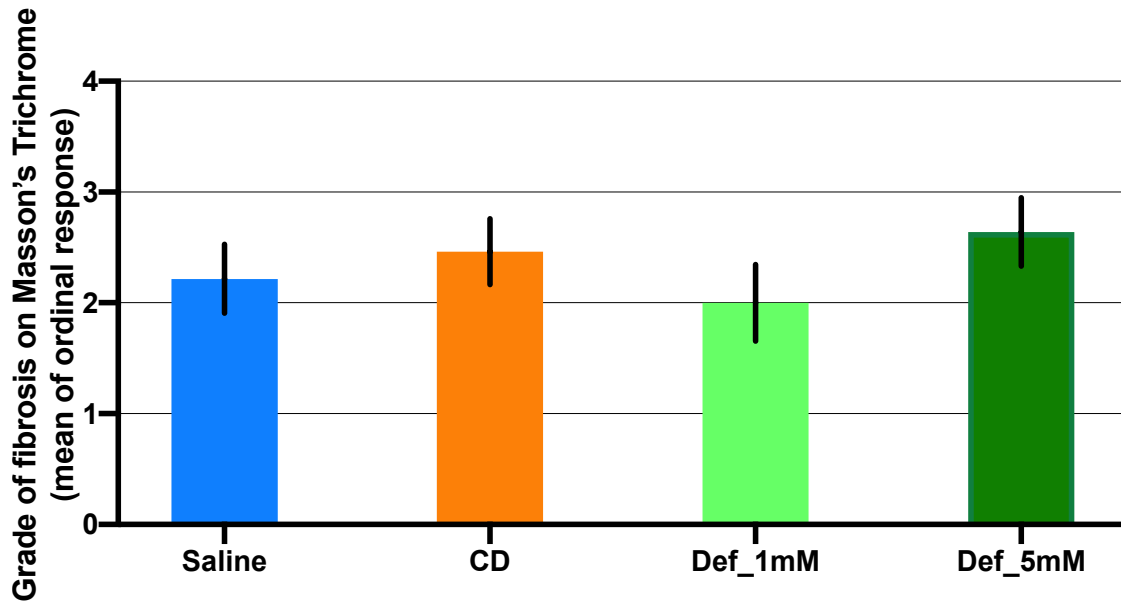


Figure 4 E Blinded Grade of Fibrosis on Masson's Trichrome staining

Figure 4A; Photographs of rat abdomen at end point depicting different grades of adhesion. I - No adhesions, II - Thin adhesion strands, III - Thich adhesions with focal attachment, IV - Thick adhesion with more broad-based planar attachment, 4B **Bar graph of blinded macroscopic grading of adhesion in a rat colon abrasion model**

Mean grade of adhesion after colon abrasion in rats treated with saline (n=12), Chitogel (CD, n=12), Chitogel with 5mM Deferiprone (Def\_5mM, n=12), Chitogel with 10mM Deferiprone (Def\_10mM, n=12), Chitogel with 20mM Deferiprone (Def\_20mM, n=12). \*\*  $p < 0.01$  compared to saline control, 4C; **Bar graph of blinded macroscopic grading of adhesion in an abrasion + enterotomy model**, mean grade of adhesion after colon abrasion + enterotomy in rats treated with saline (n=12), Chitogel (CD, n=12), Chitogel with 1mM Deferiprone (Def\_1mM, n=12), compared to saline control and Chitogel with 5mM Deferiprone (Def\_5mM, n=12), \* $p < 0.05$ , 4D; **Bar graph of Masson's Trichrome staining grading of caecal scars based on table 3**

Mean grade of fibrosis after colon abrasion in rats treated with saline (n=12), Chitogel (CD, n=12), Chitogel with 20mM Deferiprone (Def\_20mM, n=12), Chitogel with 10mM Deferiprone (Def\_10mM, n=12), Chitogel with 5mM Deferiprone (Def\_5mM, n=12).

#### Colon Abrasion with Enterotomy Model

Adhesions seen in this group of rats as compared to the abrasion model were thicker and more abundant at the suture site. The mean adhesion score of the control rats treated with saline in the colon abrasion with enterotomy group was 4.87 (CI 4.36, 5.38). Rats treated with Chitogel alone had similar adhesion scores of 4.43 (CI 3.88, 4.9) compared to control ( $p>0.05$ ). The rats treated with Chitogel in combination with 2 low dosages of Deferiprone of 5 mM and 1 mM, showed significant reductions of abdominal adhesions macroscopically with mean adhesion scores of 3.66 (CI 3.12, 4.21) and 3.69 (CI 3.06, 4.32) respectively ( $p<0.05$ ) (Fig. 4C).

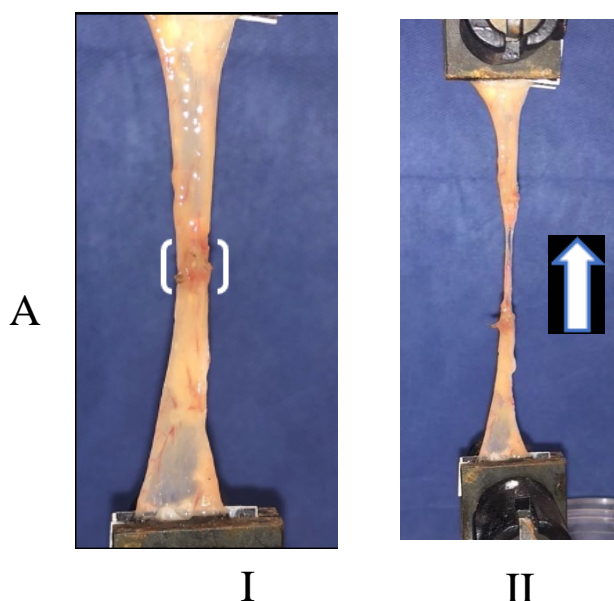
#### **Histopathology:**

Caecal adhesions and scar tissue from control rats in both the abrasion and abrasion with enterotomy models showed inflammation and mature adhesions with abundant compact collagen and few fibroblasts. The caecal scar tissue from rats treated with Chitogel and Def showed predominantly epithelioid macrophage infiltration, with phagocytosed adhesion-inciting material, with fewer multinucleated giant cells, lymphocytes and plasma cells. Masson Trichrome staining of adhesions showed a significant reduction in fibroblastic activity with reduced collagen deposition in rats treated with Chitogel with lower dosages of Def in the rat abrasion model (Def 5mM  $p<0.05$  and Def 10mM  $P<0.05$ ) (Fig. 4D). There was similar reduction of fibroblastic activity in the enterotomy model (Def 5mM & 1mM) but this was not statistically significant (Fig. 4 E).

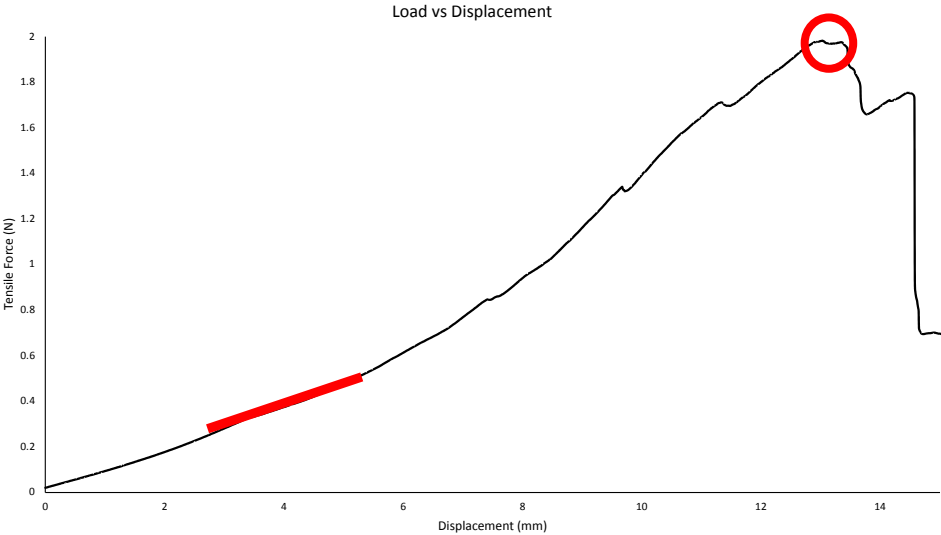
### Tensile strength Testing:

Electromechanical tensile strength testing revealed differences between samples of the localisation of tissue rupture (Supplementary Table 2). Naïve caecum colon mucosa ruptured 7/12 mid-specimen (58.3%) and 5/12 (41.7%) in the lower, thinner part of the mucosa and none in the upper, thicker part of the mucosa (Figure 5A I). In contrast, in control colon anastomosis samples, colon mucosa ruptured 2/11 mid-specimen (18%), 3/11 (27%) in the upper, thicker part of the mucosa and 6/11 (54%) in the lower, thinner part of the mucosa. Chitogel with Def 1mM and Def 5 mM treated colon anastomosis never failed within the repair zone and rupture sites were similar with 0/22 ruptures occurring mid-specimen (0% of all ruptures), 8/22 (36%) in the upper, thicker part of the mucosa and 14/22 (64%) in the lower, thinner part of the mucosa, whereas 1/12 (8%) failed at the repair zone in Chitogel alone treated wound, All enterotomy groups (i.e. control, Chitogel and Chitogel with Def 5mm), except the Chitogel with Def 1 mM group ( $p=0.142$ ), had significantly lower peak loads than the naïve tissue ( $p<0.05$ ). Def 1mM and Def 5 mM had significantly larger peak loads than Chitogel Only ( $p<0.001$  and  $p=0.049$ , respectively) (Fig 5 C). All “repaired” specimens had significantly lower stiffness than the naïve tissue ( $p<0.001$  for all) (Fig 5D).

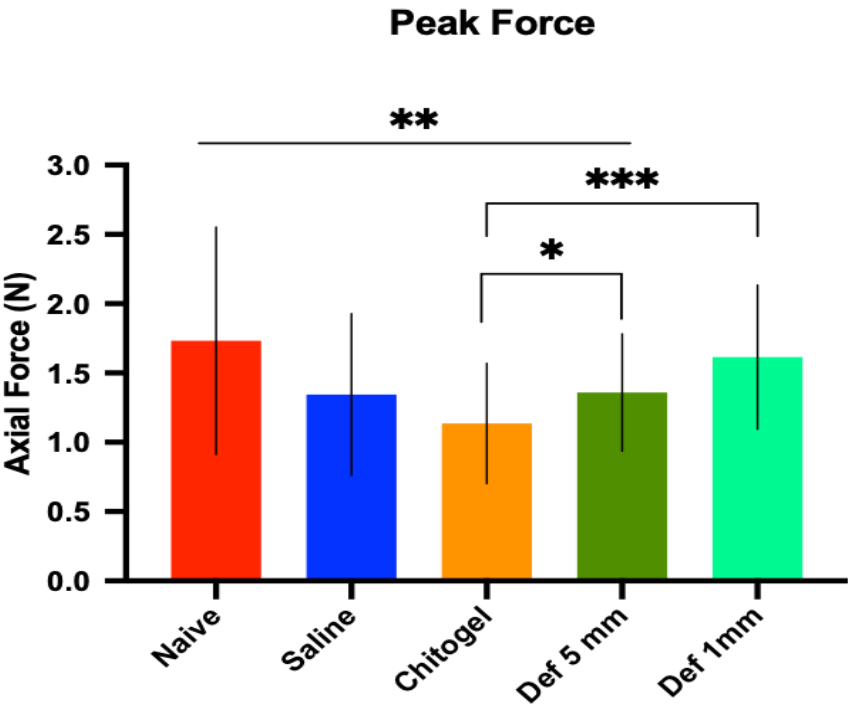
### Electromechanical tensile testing

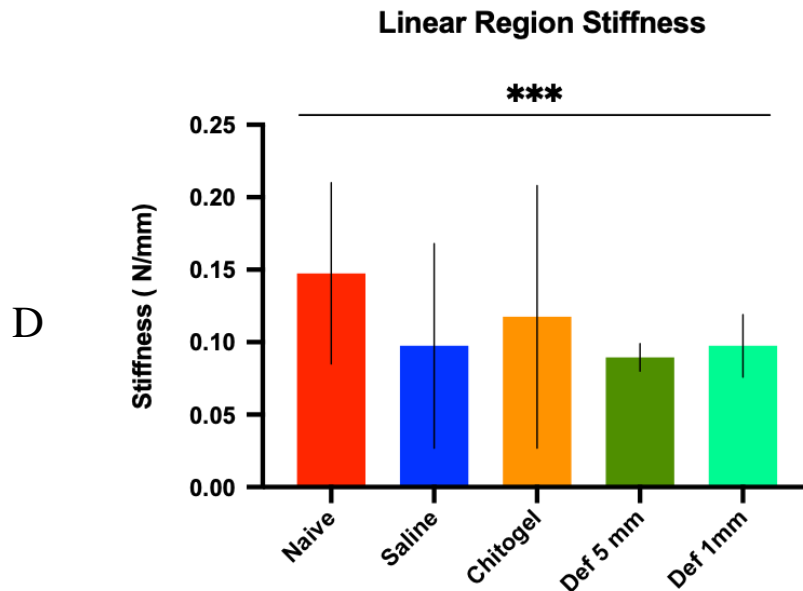


B



C





**Figure 5: Electromechanical tensile strength testing**

(A) Experimental set up showing specimen caecum split open and fixed to the Instron pneumatic arms with sutured area at its centre before being stretched (thick white arrow indicates direction of pull) for electromechanical tensile testing. (B) Load displacement graph showing value of tensile force [in Newton(N)] vs displacement [in millimetre(mm)]. The red line represents the linear region, from which the stiffness was calculated, and the red circle indicates the peak load location. (C) Bar graph of peak axial force [in Newton (N)] for the different treatment groups \*\*\* $p < 0.001$ , \*  $p < 0.05$ , (D) Bar graph of Linear region stiffness [in Newton/mm (N/mm)] of caecum in different treatment groups specimens. \*\*\*  $p < 0.001$ , compared to Naïve tissue.

**Supplementary Table 1:****Table of Adhesion Grade with Abrasion alone and Abrasion with Enterotomy**

| Macroscopic Grading of Adhesion                                |                     |      |      |         |
|----------------------------------------------------------------|---------------------|------|------|---------|
| Arm of Study                                                   | Treatment           | mean | SE   | P value |
| Abrasion only                                                  | Saline(Control)     | 3.98 | 0.33 |         |
|                                                                | Chitogel            | 3.51 | 0.29 | 0.20    |
|                                                                | Chitogel + Def 20mM | 3.64 | 0.44 | 0.53    |
|                                                                | Chitogel +Def 10mM  | 3.33 | 0.37 | 0.19    |
|                                                                | Chitogel +Def 5mM   | 2.77 | 0.29 | 0.001   |
|                                                                | Chitogel +Def 1mM   | 2.79 | 0.41 | 0.006   |
| Abrasion with Enterotomy                                       | Saline(Control)     | 4.87 | 0.26 |         |
|                                                                | Chitogel            | 4.43 | 0.28 | 0.9174  |
|                                                                | Chitogel + Def 5mM  | 3.66 | 0.28 | 0.042   |
|                                                                | Chitogel + Def 1mM  | 3.69 | 0.32 | 0.018   |
| Microscopic Grading of Fibrosis by Masson's Trichrome staining |                     |      |      |         |
|                                                                | Treatment           | mean | SE   |         |

|                          |                     |          |          |  |
|--------------------------|---------------------|----------|----------|--|
| Abrasion                 | Saline(control)     | 2.450602 | 0.197389 |  |
|                          | Chitogel            | 2.161037 | 0.205117 |  |
|                          | Chitogel + Def_20mM | 2.085894 | 0.213718 |  |
|                          | Chitogel + Def 10mM | 1.854784 | 0.195241 |  |
|                          | Chitogel + Def 5mM  | 1.819321 | 0.216551 |  |
|                          | Chitogel + Def 1mM  |          |          |  |
| Abrasion +<br>Enterotomy | _saline             | 2.476377 | 0.163667 |  |
|                          | cd                  | 2.562720 | 0.157606 |  |
|                          | def_1mM             | 2.429625 | 0.173113 |  |
|                          | def_5mM             | 2.617976 | 0.158781 |  |

**Supplementary Table 2:** Number of failures at each site for each treatment group.

Thin/thick refers to the region of the specimen in which failure occurred.

| Naïve  |      |       | Control |      |       | Chitogel Only |      |       | 1 mmol |      |       | 5 mmol |      |       |
|--------|------|-------|---------|------|-------|---------------|------|-------|--------|------|-------|--------|------|-------|
| Middle | Thin | Thick | Middle  | Thin | Thick | Middle        | Thin | Thick | Middle | Thin | Thick | Middle | Thin | Thick |
| 7      | 5    | 0     | 2       | 6    | 3     | 1             | 3    | 8     | 0      | 7    | 4     | 0      | 7    | 4     |

## Discussion:

While Chitogel has well documented anti-adhesive properties <sup>332,362,415</sup>, this study demonstrated that the addition of Deferiprone at lower concentrations of 5 mM and 1 mM to Chitogel further improved Chit gel's anti-adhesive properties. This resulted in a significant reduction in adhesions in the abdominal cavity post abdominal surgery when assessed macroscopically and microscopically. Moreover, although the adhesions produced in the positive control animals were robust due to the presence of an inducing agent, histopathological data showed reduced collagenous connective tissue in Def-Chitogel treated animals. Importantly, the addition of 1 mM and 5 mM Def to Chitogel did not reduce the strength of the scar tissue. Def 1mM and Def 5 mM treated sites never failed at the repair site and an increase in peak load was observed when compared to Chitogel alone. In fact, peak load, indicative of wound healing of enterotomy sites, was significantly higher when Def 1mM-Chitogel was applied compared to control saline treated enterotomy sites and was similar to naïve, non-operated tissue. Together, these results indicate that Def 1mM-Chitogel, apart from reducing post-operative adhesions, actively promotes wound healing of the enterotomy site. This data supports the excellent potential of Def 1mM-Chitogel as an anti-adhesion device for use after open abdominal surgery with and without enterotomy.

Wound healing after abdominal surgery is a complex process and, to a large extent, depends on the site and organs involved <sup>25</sup>. Adhesions are common after surgery on the abdominal wall, abdominal viscera, and the urogenital system <sup>288</sup>. While adhesions formed after peritoneal injury are uniquely formed by sheets of mesothelium <sup>464</sup>, most abdominal adhesions are formed by organisation of a fibrin-rich haematoma and characterised by infiltration of fibrovascular granulation tissue, the fibroblastic component laying down collagen, which forms the healed scar tissue. This process of post-surgical blood clot



organisation is further complicated and impeded by inflammation and infection. To date, there have been various strategies devised to prevent adhesions, mainly in the form of peritoneal irrigates, instillates or barriers<sup>278</sup>. The application of silastic sheets within 36 hours of surgery, for example, is able to reduce adhesion formation from 100% to 0%<sup>278</sup>.

However, although barrier systems have proven to be useful in reducing adhesions, no agent has progressed to widespread clinical use, in large part due to the lack of clinical efficacy or undesirable side effects<sup>465</sup>. Adhesion-reducing liquid barriers, such as icodextrin solution or polyethylene glycol, rely on the principle of hydro-flotation, but have not been proven useful in all situations<sup>24</sup>. Films such as oxidised regenerated cellulose<sup>278</sup> or hyaluronate carboxymethylcellulose act as a mechanical barrier, separating the operative surfaces within the abdomen. While these are solid barriers, their solubility and longevity in the abdomen remain problematic and they have limited role in laparoscopic surgery<sup>466</sup>.

Chitogel has been extensively studied in ENT surgery as a post-operative dressing in the nasal and sinus cavities<sup>44-46,60,252,467</sup>. The viscous nature of Chitogel enables it to conform to narrow spaces<sup>4,77,252</sup> and deliver anti-adhesive and anti-microbial drugs in chronic sinusitis surgery<sup>78,97</sup>. In order to prevent intra-abdominal adhesion formation, post-surgical haemorrhage, inflammation and inhibition of inflammatory cytokine-driven fibroblastic infiltration are required<sup>445</sup>. Chitogel has haemostatic<sup>44</sup>, anti-inflammatory and anti-proliferative properties<sup>80</sup>. Deferiprone's potent anti-inflammatory and inhibitory effects on fibroblast migration potentiate Chitogel's anti-adhesive properties<sup>85</sup>. Def also has inhibitory effects on Reactive Oxygen Species (ROS) generation and collagen secretion by primary fibroblasts<sup>85</sup>. This combined effect results in reduced fibrosis in the rat abdomen treated with Chitogel and Deferiprone when compared to Saline and Chitogel alone in both our abrasion and enterotomy with abrasion models. The Def release profile from Chitogel has shown that

the complete release of Def occurs within 72 h<sup>365</sup> and maximum serum levels are reached within 24 h<sup>97</sup>. These findings indicate that Def affects wound healing in the early stages of wound repair, a time when the production of ROS and associated inflammation is maximal<sup>85</sup>. Histological findings in the present study are concordant with these actions in the form of reduced fibroblastic proliferation and attenuated collagen deposition.

While the anti-fibrotic effect of some interventions such as hyaluronic acid-based films, reduce the quality of wound healing<sup>468</sup> and promote fistula formation<sup>469</sup>, the tensile strength of caecal tissue treated with Def-Chitogel in the present study was not compromised.

In the enterotomy part of this study, the antimicrobial GaPP was omitted as no microorganisms were cultured from the abdominal cavity in the control animals at day 21. Without a positive bacteriological swab at day 21, we would have been unable to show any benefit of adding GaPP. Moreover, lower Def dosages of 5 mM and 1 mM were used in this cohort because our results indicated an inverse dose response when using Def dosages of 10mM and above. A previous sheep laminectomy study similarly showed reduced anti-adhesive capacity of Def-Chitogel when Def concentrations above 20 mM were used<sup>367</sup>. The reason for this reduced anti-adhesive capacity at high-Def concentrations in Chitogel is unclear. Ramezanpour et al showed no significant toxicity when 10 mM Def was applied to primary fibroblasts and primary human nasal epithelial cells for up to 48 hours<sup>85</sup>. In that study, Def at higher concentrations of 10 and 20 mM had stronger anti-inflammatory properties than corticosteroids at clinically relevant concentrations. Whilst excessive inflammation can induce adhesions, it is well known that a low degree of inflammation is needed as part of the normal healing process after surgery<sup>19</sup>. Therefore, Def concentrations in excess of 10 mM might deregulate this balancing act resulting in a loss of beneficial anti-adhesive and wound healing properties. Reducing inflammation may also delay healing at the

level of suture lines or anastomotic sites therefore demonstration of conserved tissue-holding strength of sutures and anastomotic sites is critical for abdominal adhesion barrier devices in particular if those are to be used in indications of enterotomy. The enterotomy part of our study replicates the clinical indication of open invasive abdominal surgery, e.g., involving the removal or opening of the gut with the associated intra-abdominal bacterial contamination that occurs with such an enterotomy. The tensile strength tests performed demonstrated that Chitogel with Def concentrations of 5mM and 1 mM was safe and allowed for a normal caecal wound healing to occur. In fact, Def-Chitogel at 1 mM Def concentration resulted in anastomotic sites that had superior tissue strength than Chitogel treated animals without any significant difference with naïve rats that did not undergo abrasion/enterotomy. These results indicate that Chitogel with 1mM Def not only prevents adhesion formation but also promotes efficient healing of the enterotomy site, setting this product apart from all other marketed adhesion barrier devices. Whilst further research is needed to confirm these promising findings in large animal models of abdominal surgery, our results support the potential beneficial properties of Chitogel incorporating 1mM Def for use to prevent adhesions after abdominal surgery with enterotomy.

In conclusion, the results of this rat study demonstrated that Chitogel with 1mM Deferiprone is a safe and effective product significantly reducing abdominal adhesion formation. Confirmation of safety and anti-adhesive properties in large animal models are required prior to advancing this technology towards human clinical trials.

## 8 Chitogel with Def in Spine Surgery

**Summary:** Failed Back Syndrome is a painful condition that arises out of abnormal scarring in the spinal foramina or canal region after spinal surgery, performed to relieve pain. Pressure on the nerve roots have debilitating effect on the patients, innovative therapeutic models and regimens have been espoused to avoid scar formation and inhibit excessive tissue deposition in the narrow-confined spaces of the spinal cord. Recommended anti-adhesion regimens are based on principles of - reduction of inflammatory reaction, quick clot formation, and limited fibrin deposition<sup>470</sup>. Most of these are designed to implant a synthetic or organic material into the laminectomy site as a barrier between the exposed dura mater and surrounding muscles - Silastic, Dacron, methacrylate, bone graft, synthetic membranes and foams, free and pedicle fat grafts, and steroid agents<sup>387</sup>. There is, however, no consensus amongst spine surgeons as to the best and most effective option. Recently, surgical hydrogels have been developed and specifically marketed for their use to improve the clinical outcomes and prevent adhesions after spine surgery. FzioMed's surgical hydrogel for spine surgery<sup>471</sup>, is marketed under the brand names Oxiplex<sup>®</sup>, Oxiplex<sup>®</sup>/SP or MediShield<sup>™</sup>. It is serving as a protective physical barrier and is specifically marketed for reducing pain, lower extremity weakness, and the incidence, extent, and severity of postoperative adhesions after laminectomy. Clinical trials have shown that coating the surgical site with Oxiplex improves clinical outcomes after spine surgery<sup>377</sup>. However, due to efficacy data being discrete compared to control, no FDA approval has been granted to date despite FzioMed's repeated filings and appeals to the FDA for more than a decade.

Chitogel has demonstrated many properties that result in improved wound healing *in vitro* and *in vivo* (animal and human studies) proof-of-concept data previously<sup>417</sup>. These include haemostasis<sup>44</sup> and anti-adhesive properties<sup>49</sup>, biocompatibility, non-toxicity and also as an excellent drug delivery device in particular for hydrophilic compounds.

### **8.1.1 Paper 4: Prevention of peridural adhesions in spinal surgery:**

#### **Assessing safety and efficacy of Chitogel with Deferiprone in a**

#### **Sheepmodel**

Conducted in the Department of Otolaryngology – Head and Neck Surgery

The University of Adelaide, Adelaide, Australia,

Financial assistance provided by The University of Adelaide and The Hospital Research

Foundation under Australian Government Research Training Program Scholarship



Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: [www.elsevier.com/locate/jocn](http://www.elsevier.com/locate/jocn)

## Experimental study

## Prevention of peridural adhesions in spinal surgery: Assessing safety and efficacy of Chitogel with Deferiprone in a sheep model



Rajan Sundaresan VEDIAPPAN<sup>a</sup>, Annika Mascarenhas<sup>a</sup>, Ann Nguyen-Hoang<sup>b</sup>, Stephanie Anne Fong<sup>a</sup>, Alistair Jukes<sup>a</sup>, Katharina Richter<sup>c</sup>, Ahmed Bassiouni<sup>a</sup>, Sandy Patel<sup>d</sup>, Steve Chryssidis<sup>e</sup>, Sophia Otto<sup>b</sup>, Stephen Moratti<sup>f</sup>, Alkis James Psaltis<sup>a</sup>, Sarah Vreugde<sup>a</sup>, Peter-John Wormald<sup>a,\*</sup>

<sup>a</sup> Department of Surgery – Otolaryngology Head and Neck Surgery, The University of Adelaide, Adelaide, Australia

<sup>b</sup> SA Pathology, Royal Adelaide Hospital, Adelaide, Australia

<sup>c</sup> Department of Surgery, Richter Lab, Basil Hetzel Institute for Translational Health Research, The University of Adelaide, Adelaide, Australia

<sup>d</sup> Department of Medical Imaging, Royal Adelaide Hospital, Adelaide, Australia

<sup>e</sup> Department of Medical Imaging, Flinders Medical Center, Adelaide, Australia

<sup>f</sup> Department of Chemistry, Otago University, Dunedin, New Zealand

## ARTICLE INFO

## Article history:

Received 6 September 2019

Accepted 16 December 2019

## Keywords:

Back pain

Epidural adhesion

Fibrosis

Chitogel

Failed Back surgery syndrome

## ABSTRACT

**Introduction:** Spinal laminectomy is a common procedure performed to relieve neural compression in patients suffering from myelopathy or radiculopathy. However, up to 40% of patients suffer from persistent post-operative pain and disability, a condition known as Failed Back Surgery Syndrome (FBSS). Excessive scarring in the surgical bed is implicated as a cause. Hydrogels have been proposed to prevent adhesion formation post-laminectomy; however, their efficacy has not been proven. This study uses Chitogel complexed with the iron chelator Deferiprone (Def) to prevent adhesion formation in a sheep laminectomy model.

**Material & methods:** Fifteen Adult Merino sheep (Ovis Aries, 1–5 yrs old) underwent laminectomy at lumbar levels 1–5 and had hydrated aluminum silicate (kaolin) applied to promote adhesion formation. Subjects were randomised to receive at each laminectomy level no-treatment control, Chitogel, Chitogel with Def at 20 mM or 40 mM or Carboxy-methyl-cellulose and Polyethylene oxide (CMC/PEO) gel. The animals were recovered for 3 months post-surgery, followed by assessment with Magnetic Resonance Imaging (MRI) and histopathology of the spinal tissues for evaluating the presence and extent of adhesions.

**Results:** MRI and Histology assessment indicated that Kaolin induced severe inflammation with adhesion formation. Chitogel with and without 20 mM Def decreased inflammation ( $p < 0.01$ ) and trended to reduce adhesions ( $p < 0.1$ ). Chitogel with Def 40 mM was not significantly dis-similar to CMC/PEO and did not reduce inflammation or adhesions compared to no-treatment control.

**Conclusion:** Chitogel in combination with Def 20 mM is safe and effective in decreasing the inflammatory process and may possibly reduce post-operative adhesions following laminectomy.

Crown Copyright © 2019 Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

The prevalence of herniated disc associated with back pain and/or radicular leg pain is 1–3% in western societies requiring laminectomy with decompression of neural elements in 10% of patients [1]. >800,000 spine surgeries occur annually globally to provide relief, however in up to 40% of patients significant

post-operative back pain occurs [2,3]. This is sometimes referred to as failed back surgery syndrome (FBSS). Based on Australian Bureau of Statistics data, FBSS occurs in up to 6,660 of the 22,200 patients undergoing spinal surgery each year in Australia [4]. Epidural adhesions, formed after surgery, contribute to this pain through tension on neural elements in more than 80% of FBSS patients with a direct relationship between the severity of adhesions and pain scores [5]. Wound healing after tissue injury involves 4 major coordinated and regulated steps: haemostasis, inflammation, proliferation, and remodelling. Different factors can interfere with these steps, causing improper or impaired

\* Corresponding author at: Department of Surgery – Otolaryngology, Head & Neck Surgery, The Queen Elizabeth Hospital, 28, Woodville Road, Woodville south-5011, Adelaide, Australia

E-mail address: [peterj.wormald@adelaide.edu.au](mailto:peterj.wormald@adelaide.edu.au) (P.-J. Wormald).

<https://doi.org/10.1016/j.jocn.2019.12.042>

0967-5868/Crown Copyright © 2019 Published by Elsevier Ltd. All rights reserved.

## Statement of Authorship

|                     |                                                                                                                                                                                                                                                                |
|---------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title of Paper      | Prevention of peridural adhesions in spinal surgery: Assessing safety and efficacy of Chitogel with Deferiprone in a Sheep model                                                                                                                               |
| Publication Status  | <input checked="" type="checkbox"/> Published <input type="checkbox"/> Accepted for Publication<br><input type="checkbox"/> Submitted for Publication <input type="checkbox"/> Unpublished and Unsubmitted work written in manuscript style                    |
| Publication Details | Journal of Clinical Neuroscience<br>The official journal of the Neurosurgical Society of Australasia, the Australian and New Zealand Society for Neuropathology, the Taiwan Neurosurgical Society, and the Asian Australasian Society of Neurological Surgeons |

### Principal Author

|                                      |                                                                                                                                                                                                                                                                                                |      |            |
|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|------------|
| Name of Principal Author (Candidate) | Rajan Sundaresan VEDIAPPAN                                                                                                                                                                                                                                                                     |      |            |
| Contribution to the Paper            | Project Design, data collection, sample processing, Image analysis, statistical analysis and manuscript preparation                                                                                                                                                                            |      |            |
| Overall percentage (%)               | 75%                                                                                                                                                                                                                                                                                            |      |            |
| Certification:                       | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |      |            |
| Signature                            |                                                                                                                                                                                                                                                                                                | Date | 17.12.2019 |

### Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate to include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

|                           |                                                                                  |      |          |
|---------------------------|----------------------------------------------------------------------------------|------|----------|
| Name of Co-Author         | Annika Mascarenhas                                                               |      |          |
| Contribution to the Paper | Project Design, data collection, Image analysis and manuscript preparation - 10% |      |          |
| Signature                 |                                                                                  | Date | 17.12.19 |

|                                                                              |                                                                                    |      |            |
|------------------------------------------------------------------------------|------------------------------------------------------------------------------------|------|------------|
| Name of Co-Author                                                            | Ann Nguyen-Hoang                                                                   |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, sample processing and manuscript preparation - 2% |      |            |
| Signature                                                                    |                                                                                    | Date | 17.12.19   |
| Name of Co-Author                                                            | Stephanie Anne Fong                                                                |      |            |
| Contribution to the Paper                                                    | Data collection and manuscript preparation -1%                                     |      |            |
| Signature<br>(by Principal Supervisor with<br>accepted format of authorship) |                                                                                    | Date | 17.12.19   |
| Name of Co-Author                                                            | Alistair Jukes                                                                     |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -1%     |      |            |
| Signature<br>(by Principal Supervisor with<br>accepted format of authorship) |                                                                                    | Date | 17.12.19   |
| Name of Co-Author                                                            | Katharina Richter                                                                  |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation - 1%    |      |            |
| Signature                                                                    |                                                                                    | Date | 17.12.2019 |
| Name of Co-Author                                                            | Ahmed Bassiouni                                                                    |      |            |
| Contribution to the Paper                                                    | Data collection, Image analysis and manuscript preparation -1%                     |      |            |
| Signature                                                                    |                                                                                    | Date | 17.12.2019 |
| Name of Co-Author                                                            | Sandy Patel                                                                        |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation - 1%    |      |            |
| Signature                                                                    |                                                                                    | Date | 17.12.2019 |
| Name of Co-Author                                                            | Steve Chryssidis                                                                   |      |            |



|                                                                              |                                                                                |      |            |
|------------------------------------------------------------------------------|--------------------------------------------------------------------------------|------|------------|
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -1% |      |            |
| Signature<br>(by Principal Supervisor with<br>accepted format of authorship) |                                                                                | Date | 17.12.2019 |
| Name of Co-Author                                                            | Sophia Otto                                                                    |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -1% |      |            |
| Signature                                                                    |                                                                                | Date | 17.12.2019 |
| Name of Co-Author                                                            | Stephen Moratti                                                                |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -1% |      |            |
| Signature                                                                    |                                                                                | Date | 17.12.2019 |
| Name of Co-Author                                                            | Alkis James Psaltis                                                            |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -1% |      |            |
| Signature                                                                    |                                                                                | Date | 17.12.2019 |
| Name of Co-Author                                                            | Sarah Vreugde                                                                  |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -2% |      |            |
| Signature                                                                    |                                                                                | Date | 17.12.2019 |
| Name of Co-Author                                                            | Peter-John Wormald                                                             |      |            |
| Contribution to the Paper                                                    | Project Design, data collection, Image analysis and manuscript preparation -2% |      |            |
| Signature                                                                    |                                                                                | Date | 17/12/19   |

## **Prevention of peridural adhesions in spinal surgery: Assessing safety and efficacy of Chitogel with Deferiprone in a Sheep model**

Rajan Sundaresan VEDIAPPAN<sup>1</sup>, Annika Mascarenhas<sup>1</sup>, Ann Nguyen-Hoang <sup>2</sup>, Stephanie Anne Fong<sup>1</sup>, Alistair Jukes<sup>1</sup>, Katharina Richter<sup>3</sup>, Ahmed Bassiouni<sup>1</sup>, Sandy Patel<sup>4</sup>, Steve Chryssidis<sup>5</sup>, Sophia Otto<sup>2</sup>, Stephen Moratti<sup>6</sup>, Alkis James Psaltis<sup>1</sup>, Sarah Vreugde<sup>1</sup>, Peter-John Wormald <sup>1</sup>

<sup>1</sup>Department of Surgery - Otolaryngology Head and Neck Surgery, The University of Adelaide, Adelaide, Australia

<sup>2</sup>SA Pathology, Royal Adelaide Hospital, Adelaide, Australia

<sup>3</sup>Department of Surgery, Richter Lab, Basil Hetzel Institute for Translational Health Research, The University of Adelaide, Adelaide, Australia

<sup>4</sup>Department of Medical Imaging, Royal Adelaide Hospital, Adelaide, Australia

<sup>5</sup>Department of Medical Imaging, Flinders Medical Center, Adelaide, Australia

<sup>6</sup>Department of Chemistry, Otago University, Dunedin, New Zealand

Conflict of Interest: PJW and SM are shareholders in Chitogel. PJW and SV are patent holders of the combination product intellectual property.

**Corresponding Author:**

Professor Peter John Wormald

The University of Adelaide

Department of Surgery - Otolaryngology, Head & Neck Surgery

The Queen Elizabeth Hospital

28, Woodville Road, Woodville south-5011

Adelaide, South Australia

Contact: peterj.wormald@adelaide.edu.au

Ph: (+61 8) 8222 7158 Fax: (+61 8) 8222 7419

Author Contributions: RSV, AM, AJ, ANH, SF, KR, AB and MT were involved in the conduct of the study, collection of data and interpretation of the result with drafting and editing the manuscript. SP, SC & SO were involved in supervision, Interpretation of data, drafting and editing the manuscript. PJW, SV, SM, & AJP were involved in designing the study, supervision, Interpretation of data, drafting and editing the manuscript and final permission for submission.

This work was supported by an NHMRC Development grant (APP1153663) to PJW and SV

Abstract:

**Introduction:** Spinal laminectomy is a common procedure performed to relieve neural compression in patients suffering from myelopathy or radiculopathy. However, up to 40% of patients suffer from persistent post-operative pain and disability, a condition known as Failed Back Surgery Syndrome (FBSS). Excessive scarring in the surgical bed is implicated as a cause. Hydrogels have been proposed to prevent adhesion formation post-laminectomy; however, their efficacy has not been proven. This study uses Chitogel complexed with the iron chelator Deferiprone (Def) to prevent adhesion formation in a sheep laminectomy model.

**Material & Methods:** Fifteen Adult Merino sheep (Ovis Aries, 1-5 yrs old) underwent laminectomy at lumbar levels 1-5 and had hydrated aluminium silicate (kaolin) applied to promote adhesion formation. Subjects were randomised to receive at each laminectomy level no-treatment control, Chitogel, Chitogel with Def at 20mM or 40mM or Carboxy-methylcellulose and Polyethylene oxide (CMC/PEO) gel. The animals were recovered for 3 months post- surgery, followed by assessment with Magnetic Resonance Imaging (MRI) and histopathology of the spinal tissues for evaluating the presence and extent of adhesions.

**Results:** MRI and Histology assessment indicated that Kaolin induced severe inflammation with adhesion formation. Chitogel with and without 20 mM Def decreased inflammation ( $p < 0.01$ ) and trended to reduce adhesions ( $p < 0.1$ ). Chitogel with Def 40mM was not significantly dis-similar to CMC/PEO and did not reduce inflammation or adhesions compared to no-treatment control.

**Conclusion:** Chitogel in combination with Def 20 mM is safe and effective in decreasing the inflammatory process and may possibly reduce post-operative adhesions following laminectomy.

Key words: Back pain, Epidural adhesion, Fibrosis, Chitogel , Sheep laminectomy and Failed Back surgery syndrome

Acknowledgements:

Tim Kuchel, Adam O'Connell, Sundaraj Perumal, Loren Matthews and team at LARIF Peter McNeil at the RAH Anatomical Pathology Laboratory

KR acknowledges CJ Martin Biomedical Early Career Fellowship (NHMRC #1163634) by the National Health and Medical Research Council, Australia.

**Introduction:**

The prevalence of herniated disc associated with back pain and/or radicular leg pain is 1-3% in western societies requiring laminectomy with decompression of neural elements in 10% of patients<sup>472</sup>. More than 800,000 spine surgeries occur annually globally to provide relief, however in up to 40% of patient's significant post-operative back pain occurs<sup>387,473</sup>. This is sometimes referred to as failed back surgery syndrome (FBSS). Based on Australian Bureau of Statistics data, FBSS occurs in up to 6,660 of the 22,200 patients undergoing spinal surgery each year in Australia<sup>382</sup>. Epidural adhesions, formed after surgery, contribute to this pain through tension on neural elements in more than 80% of FBSS patients with a direct relationship between the severity of adhesions and pain scores<sup>383</sup>. Wound healing after tissue injury involves 4 major coordinated and regulated steps: haemostasis, inflammation, proliferation, and remodelling. Different factors can interfere with these steps, causing improper or impaired wound healing. Central to this is an increased inflammatory response with recruitment of polymorphonuclear neutrophils<sup>54</sup> to the injury site. These cells help in the clearance of pathogens and foreign particles but also lead to tissue injury with the generation of Reactive Oxygen Species (ROS)<sup>474</sup>, excessive migration of fibroblasts and collagen deposition. These processes may result in excessive scarring or adhesion formation and can be exacerbated by pathological processes such as infection, inflammation and hematoma formation<sup>391</sup>. The extent of adhesion formation is determined mainly during the first week, and in particular the first 48 hours after the initial injury<sup>25</sup>. Numerous strategies have been tested to reduce adhesion formation post spinal surgery, however, to date, no therapeutic approaches have been wholly successful and there are no Food and Drug Administration (FDA) approved marketed devices available for this indication<sup>381,386,387</sup>.

Chitogel is proposed to reduce adhesions by acting not only as a barrier between adjacent surfaces, but also a haemostatic material and carrier for Deferiprone (Def).

Chitogel is a hydrogel product of naturally occurring Chitin in succinyl form cross-linked with dextran aldehyde powder that has been dissolved in sodium phosphate buffer solution. It has proven effects on enhancing haemostasis<sup>44</sup>, mucosal wound healing<sup>252</sup>, significant anti-adhesive properties<sup>332</sup> and is FDA approved as haemostatic adhesion preventing post-operative dressing in sino-nasal surgery<sup>46,475</sup>. It has also been shown to have protective properties in epidural spinal surgery and is safe when applied to brain tissue<sup>417</sup>. Hydrogels have controlled degradation rates and optimal biocompatibility with living tissues chemically, mechanically and electrically<sup>476</sup>. This property along with their ability to concurrently act as scaffolds to carry and deliver drugs, allows optimal promotion of regeneration of tissues and progressive wound healing.<sup>477</sup>

Deferiprone, an iron chelator used for the treatment of thalassemia<sup>81</sup>, has been shown to reduce inflammation and reactive oxygen species with a reduction in the migration and proliferation of primary fibroblasts *in vitro* in a time and dose dependent manner<sup>85</sup>. Deferiprone is released from Chitogel within 48 to 72 hours and has the potential of localized therapeutic effects when applied topically at this crucial time point in wound healing<sup>365</sup>. Hence, this study aimed to investigate the combined effects of Chitogel and Deferiprone to prevent epidural adhesions after laminectomy as well as determining its safety in a multilevel sheep laminectomy model.

## **Materials and Methods:**

### **Study design:**

The University of Adelaide and South Australian Health and Medical Research Institute (SAHMRI) Ethics Committee approved the study to be conducted at the Large Animal Research & Imaging Facility Node (LARIF) (SAHMRI: SAM300).

This was a prospective double-blinded, randomised controlled study using a sheep laminectomy model, where all sheep underwent a 5-level laminectomy procedure.

#### **Animals and Materials:**

15 male merino sheep, 1-5 years of age were used. Following acclimatisation and fasting overnight, sheep underwent general anaesthesia (GA) with intravenous Ketamine and Diazepam for induction and Isoflurane for maintenance. Each animal was placed prone with pressure points adequately protected on a modified beanbag as described<sup>417</sup>.

#### **Chitogel**

Chitogel was made up of a combination of three components: 5% succinyl-chitosan, 0.3% phosphate buffer and 3% dextran aldehyde (Chitogel, Wellington, NZ). The components are manufactured and sterilized by Chitogel. All stocks were stored at room temperature. To prepare Chitogel, dextran aldehyde (0.3 g) was dissolved in 10 mL of phosphate buffer then mixed with succinyl chitosan solution (0.5 g in 10 mL buffer) using sterile technique.

#### **Deferiprone**

Deferiprone (3-hydroxy-1,2-dimethylpyridin-4(1H)-one) (Sigma-Aldrich, St Louis, USA) was stored at room temperature.

#### **Preparation of Chitogel-Deferiprone**

Deferiprone (Def, 20 mM or 40mM) was diluted in 10 mL of 0.3% phosphate buffer under sterile conditions the day prior to use. This prepared solution was then used to dissolve dextran aldehyde (0.3 g) prior to mixing with succinyl chitosan solution (0.5 g) using sterile techniques.



Preparation of CMC/PEO (Carboxymethylcellulose and polyethylene oxide)

There is only one product (not FDA-approved) that is used for the prevention of adhesions worldwide. This is a gel and is available commercially and sold as Oxiplex (Oxiplex, FzioMed, San Luis Obispo, CA, USA)

**Surgical procedure:**

In an aseptic manner, 20 cm long posterior spinal approach midline incision was made over the spinous processes of each sheep and a sub-periosteal midline dissection made to expose the laminae by monopolar electrocautery. The spinous processes were removed at L1-L5 levels in an intersegmental pattern and laminae removed with combination of high-speed drill and rongeurs. The dorsal surface of the spinal dura was exposed approximately 2 cm X 1 cm wide and 0.5 g Kaolin (Aluminium Silicate Hydroxide,  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ , Sigma-Aldrich, St. Louis, Missouri, United States), an adhesion-inducing agent<sup>451</sup>, was mixed with 2 ml normal saline and placed on the intact dura. Following Kaolin application, each site was randomized to receive nothing (control) or 2ml of Chitogel, Chitogel with Def 20 mM, Chitogel with Def 40 mM or CMC/PEO. The wound was then closed in a multilayered fashion to eliminate surgical dead-space with 3-0 Polyglactin sutures. Pain relief was given in the form of Bupivacaine with adrenaline 0.5% as tissue infiltration immediately post-operatively and with Carprofen 1ml/25mg intraoperatively, immediately post-operatively and further as required following veterinary advice. Additionally, a transdermal Fentanyl patch (2mcg/kg/hr) was placed on the sheep 24 hours prior to surgery and then replaced after surgery on the 3rd day as needed. Antibiotic cover in the form of subcutaneous infiltration of Cephalosporin (Excenel<sup>®</sup> RTU EZ, Zoetis, Parsippany, NJ, USA) 0.3mL/10kg once daily was given pre-operatively and post-operatively for 48hrs.

### **Post-operative monitoring:**

Animals were recovered, and post-operative follow-up done for 48 hrs inside pens and monitored with CCTV every 2 hrs. Neurological examinations were carried out to assess the general condition of the sheep (food/fluid intake, temperature, teeth grinding, ground pawing and bleeding or other discharge at the wound). Basic neurological function assessment (pupillary response, circular walking, knuckling of the hind limbs and head movement to sound) were looked for every 2 hours after surgery. At the 48h post-operative mark, the animals were moved to the post-operative yards for 1 week, where they underwent once daily blood test (to determine Def levels) and observations 4 times per day. Animals were moved to a small open paddock for free roaming after 1 week for the remainder of the three months with twice daily monitoring as per scheduled blood testing, daily for the first week and then weekly for the next 3 months. After three months, the animals were brought back to the holding pens for 2 days acclimatisation with a partner at all times. Under general anaesthesia MRI of the spine was done for assessing adhesions after which the animals were humanely killed.

### **Blood examinations:**

Blood examinations included Full Blood Examination (FBE), Urea Electrolytes and Creatinine (UEC), Liver Function Tests (LFT) and Iron studies at 24h, 1 week and 1 month post op. High performance liquid chromatography (HPLC) analysis was used to determine Def levels in plasma as follows: Baseline (time of induction, T0) Deferiprone levels at T0, T0.5 hrs, T1 hrs, T2 hrs, T4 hrs, T8 hrs, T12 hrs, T24 hrs then daily for 1 week, then weekly for weeks 2, 3 and 4.

### **Quantification of Deferiprone in sheep plasma samples**

Plasma samples were analysed for Def using HPLC on a Shimadzu UFLC XR (Shimadzu Cooperation, Kyoto, Japan) as previously described<sup>97</sup>. For the quantification of Def, 250 µl plasma was mixed with 750 µl methanol (HPLC grade, Merck, Darmstadt, Germany). Following vortexing for 1 min, the samples were centrifuged for 4 min at 14,800 rpm at room temperature (Eppendorf 5804R, Eppendorf, Hamburg, Germany). A Phenomenex Synergi 4 µm Fusion-RP LC column coupled to a security guard cartridge (Phenomenex, Lane Cove, NSW, Australia) was used to quantify 50 µl of the clear supernatant using methanol/0.1 M orthophosphate buffer pH 7.2 (15%: 85%) as mobile phase at a flow rate of 2.0 ml/min. The concentration of Deferiprone was detected at 280 nm and calculated against a standard curve ranging from 1.0 to 10.0 µg/ml Deferiprone ( $R^2 > 0.983$ ).

**Imaging:**

At the end of three months, animals underwent Magnetic Resonance Imaging of the spine (MRI) using a Siemens Skyra 3T (Erlangen, Germany). T1 sagittal and transverse spin echo and T2 fast spin echo of the whole spine was made.

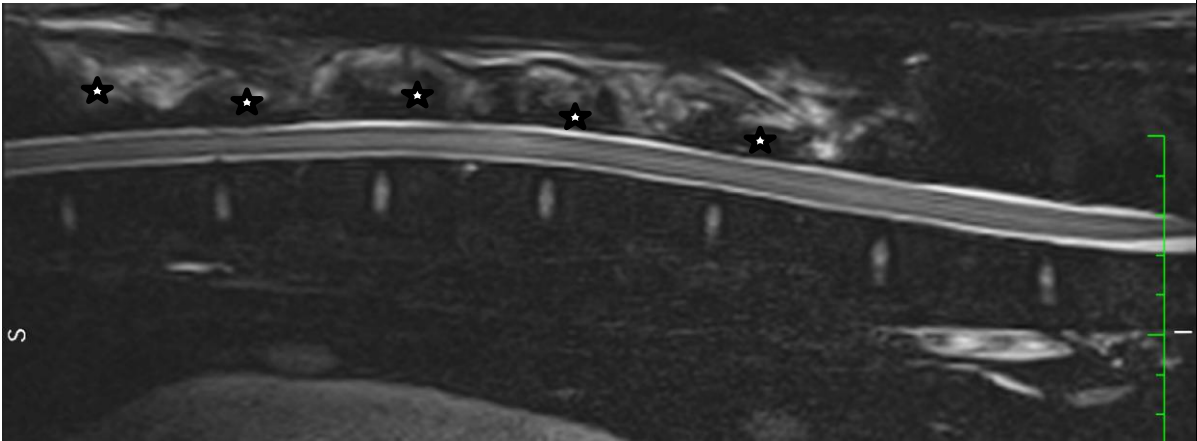
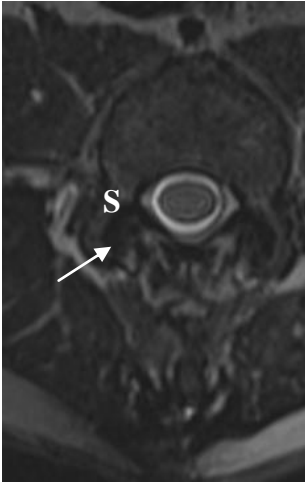
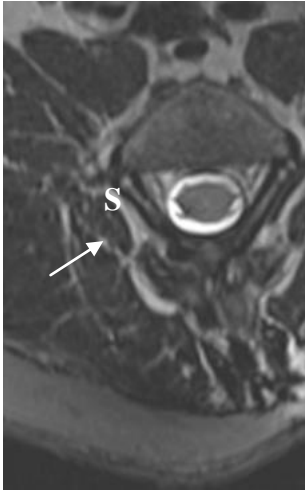


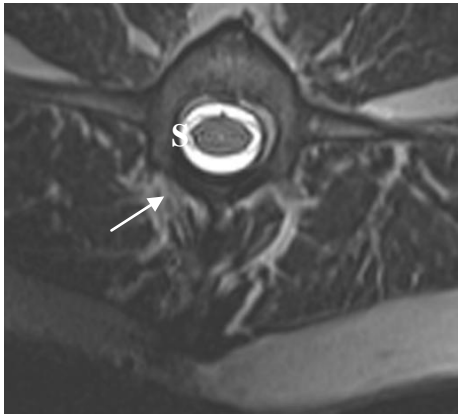
|                                                                                                                                                        |                                                                                                                                         |                                                                                                                    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------|
| <div>A</div> <div></div> <div>MRI after treatment and 12 weeks</div> |                                                                                                                                         |                                                                                                                    |
| <div></div> <div>Grade 0</div>                                       | <div><div>B</div><div></div><div>Grade 1</div></div> | <div></div> <div>Grade 2</div> |
| <div></div> <div>Grade 3</div>                                      | <div></div> <div>Grade 4</div>                      |                                                                                                                    |

Fig. 1. Radiological scheme for grading of Adhesions on Magnetic Resonance Imaging (MRI). (A) MRI images of the dorsal spine of sheep in sagittal view. Spinal cord seen as railroad appearance (S), post laminectomy scar X 5 (\*), (B) Axial images of the spinal column with spinal cord represented as (S) and the scar over the epidural layer indicated by the arrow. Grade 1 – no abnormalities, Grade 2 – abnormalities on < 8 slices, Grade 3 – abnormalities on > 8 slices but less than 1 mm thick, Grade 4 – abnormalities on > 8 slices and > 1 mm thick.

The spine sequences are as followed:

- a. T2\_Space\_Sagital (T2 anatomy); Voxel size: 0.9x0.9x0.9 mm
- b. T2\_Space\_STIR\_Sagital (T2 anatomy, fat suppressed); Voxel size: 0.9x0.9x0.9 mm
- c. T1\_Vibe\_Axial (T1 anatomy); Voxel size: 0.6x0.6x2.0 mm
- d. T2\_Space\_Axial (T2 anatomy); Voxel size: 0.6x0.6x2.0 mm
- e. T2\_Vibe\_Axial (T1 anatomy); Voxel size: 0.6x0.6x2.0 mm
- f. T2\_me2d\_Axial (Bleed Sequence); Voxel size: 0.4x0.4x3.0 mm

Fibrosis was scored by assessing the hypo-intense area (granulation tissue) in the epidural region utilizing the scoring system used by Rajiv et al <sup>417</sup>. Each level was divided into 15 slices, Grade 1 – no abnormalities, Grade 2 – abnormalities on <8 slices, Grade 3 – abnormalities on > 8 slices but less than 1mm thick, Grade 4 – abnormalities on > 8 slices and > 1mm thick (Fig 1 A & B). Following MRI, the sheep underwent euthanasia at 12 weeks.

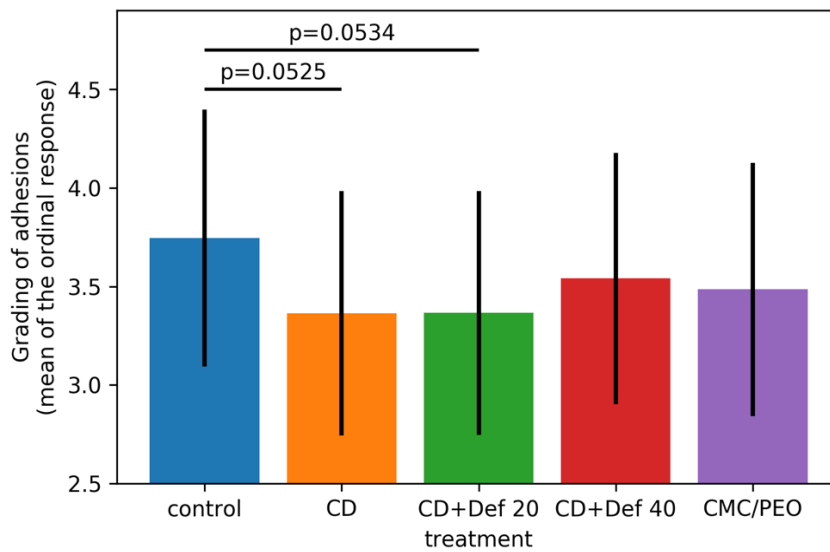


Fig. 2. Results of radiology grading of epidural scars on MRI. Graph showing the radiological score of adhesions assessed via MRI. Fibrosis was scored by assessing the hypo-intense area (granulation tissue) in the epidural region utilizing the scoring system used by Rajiv et al (16).

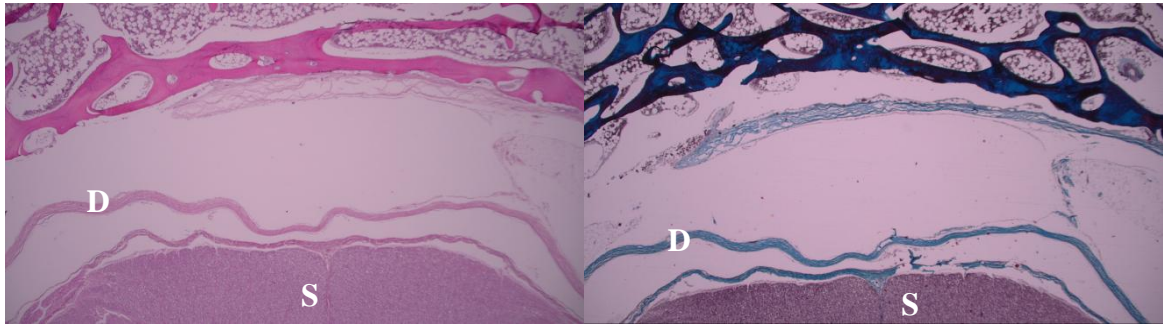
### Histopathology examination:

The sheep spines were removed en bloc and separated at each treatment level before fixation in 10% formalin. The vertebral bodies were mechanically removed prior to decalcification in a solution of 9.5% nitric acid in 1% EDTA.

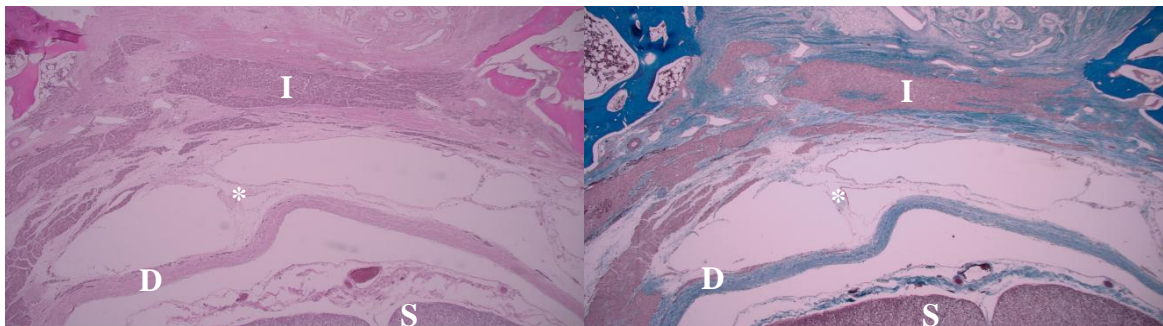
A representative section of each laminectomy site was embedded in paraffin wax and at least two 4 µm sections per defect were assessed with haematoxylin and eosin (H&E) and Masson's Trichrome (MT) stains. Each level was assessed by 1 pathologist and 1 pathology registrar who were blinded to the treatment given.

Parameters assessed included a descriptive, qualitative assessment of the epidural scar and extent of inflammation. The method used to describe the quality of adhesions was based on a modified version of a method described by Richard et.al<sup>478</sup> using MT stains (Fig 3 A-E),

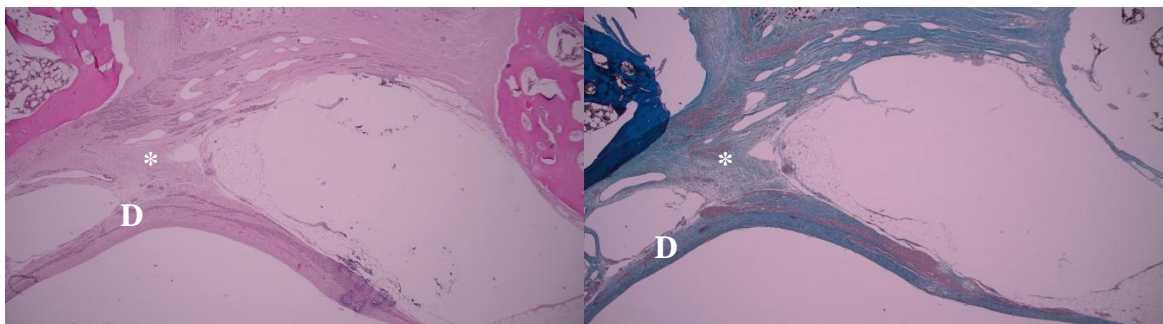
A. No treatment sheep



B. Score 1 fibrosis (mostly loose) – 10% adhesion

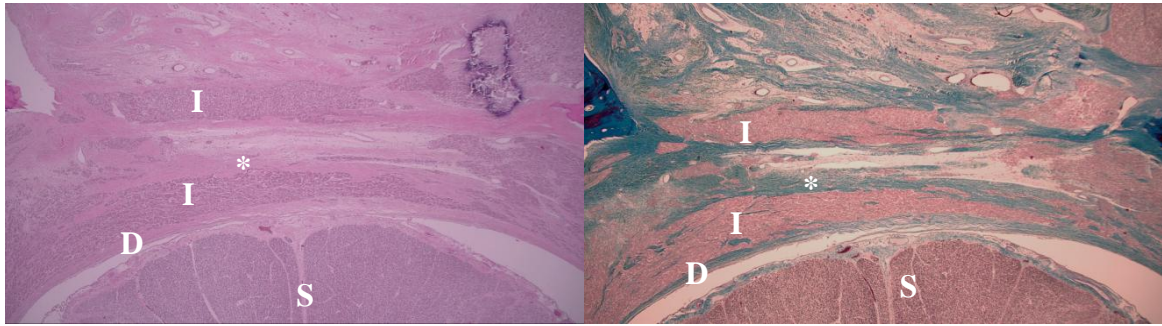


C. Score 2 fibrosis (loose to moderate) – 20% adhesion





D. Score 3 fibrosis (moderate to dense) – 100% adhesion



E. Score 4 fibrosis (dense) – 100% adhesion

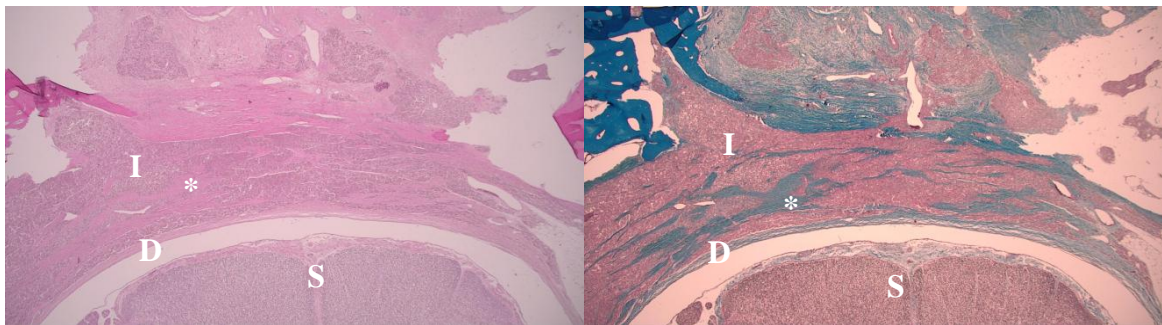


Fig 3. Histological scheme for Grading Adhesion. H&E (left) and MT (right) stained sections demonstrating the density of fibrosis corresponding to each grade. (A) Spine from a normal sheep for comparison, showing the posterior spinous process at the top and spinal cord at the bottom, with the loose dura mater in between. (B) Grade 1 fibrosis the adhesions are loose and delicate. (C) Grade 2 fibrosis – the scar is more established but still loose to moderate in density, with a pale quality on MT. (D) Grade 3 fibrosis – moderate to dense fibrosis; note the presence of chronic, foreign-body type inflammation within the scar (pink on MT). (E) Grade 4 fibrosis – the fibrosis is more dense compared to (D). S - Spinal cord, D -dura, \* - adhesion & I – inflammation.

where the epidural scar was characterised as mostly loose (score 1), loose to moderate (score 2), moderate to dense (score 3), and dense (score 4). Assessment of the post-surgical inflammation was determined in the area within the laminectomy defect site and defined as a



percentage of that area that was occupied by a chronic, foreign body-type inflammatory cell infiltrate.

### **Statistical Analysis:**

All statistics were performed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria) through the Jupyter notebook interface. The R package "ordinal" was used for ordinal regression. The "clmm" function was used to fit a Cumulative Link Mixed Models with the Laplace approximation. The semi-quantitative adhesion scores both on MRI and on H&E staining, were specified as the ordinal outcome variables. To control for the sheep variable, and the Radiology scorer variable when appropriate, they were assigned as random-effects covariates in the model. The means of the ordinal response (interpreted as a numeric value from 1 to the number of classes) were calculated and post-hoc pairwise contrasts for each pair of levels of the treatment variable were compared using the "emmeans" package<sup>442</sup>. A p-value of <0.05 was considered statistically significant.

### **Results:**

#### **Clinical evaluation**

Of the 15 sheep that underwent surgery, 13 recovered, completing 12 weeks without any neurological deficit. 2 sheep developed neurological deficits in the hind limbs within 6-12 hrs after surgery. They were unable to recover with systemic steroid treatments and observation for 48 hrs and were subsequently euthanized. On emergency post-operative MRI, both sheep showed the presence of intrathecal blood, which was thought to be due to surgical trauma. The 13 remaining sheep had normal neural functions for 12 weeks and underwent euthanasia after MRI under general anaesthesia.

### **Blood examination**

FBE, UEC, LFT did not show any significant changes from baseline in any of the sheep tested. HPLC analysis showed trace amounts of plasma Def concentrations; the maximum Def concentration was reached after 12 hours (0.62 µg/ml Def). After 7 days the Def plasma concentration decreased to 0.16 µg/ml and after 3 weeks Def was not detected anymore (Supplementary Fig 1).

### **Adhesion Scores by MRI**

MRI results were scored by 2 radiologists and 2 neurosurgeons for each of the individual laminectomy sites for 13 sheep. Assessors were blinded to the treatments given. Control sites had the highest mean adhesion grade of 3.74 [CI 3.09, 4.39]. A reduction in adhesion scores was seen for the treatment with Chitogel with a mean adhesion grade of 3.364 [CI 2.74, 3.98] and for the Chitogel + Def 20 mM treatment with a mean adhesion grade of 3.364 [CI 2.74, 3.98] respectively in comparison to no-treatment control ( $p=0.0525$  and  $P= 0.0534$  respectively). Mean adhesion scores were not significantly dis-similar for Chitogel + Def 40 mM (3.53, [CI 2.9, 4.1]) and CMC/PEO (3.484, [CI 2.8, 4.1]) and were similar to control ( $P>0.1$ ).

### **Adhesion scores by histology**

The histological evaluation was performed by 1 consultant pathologist and 1 pathology registrar blinded to the treatment. Parameters assessed included a qualitative description of the epidural scar graded as ordinal scale (histopathology adhesion score) and extent of inflammation as percentage within the surgical site. Mean histopathology adhesion scores in the no-treatment control sites were 2.4 [CI 2.1, 2.7]. Chitogel with Deferiprone 20 mM (2.15 [CI 1.94, 2.52]) and Chitogel with Deferiprone 40mM (2.06 [CI 1.8,2.3]) treated sites had the

lowest mean adhesion score, however, this reduction compared to control was not significant ( $P>0.05$ ). Mean adhesion scores were similar for Chitogel (2.23 [CI 1.94, 2.52]) and CMC/PEO (2.23 [CI 1.94,2.52]) and not statistically different to control or to Chitogel with Deferiprone 20 mM or 40 mM ( $p>0.05$ ).

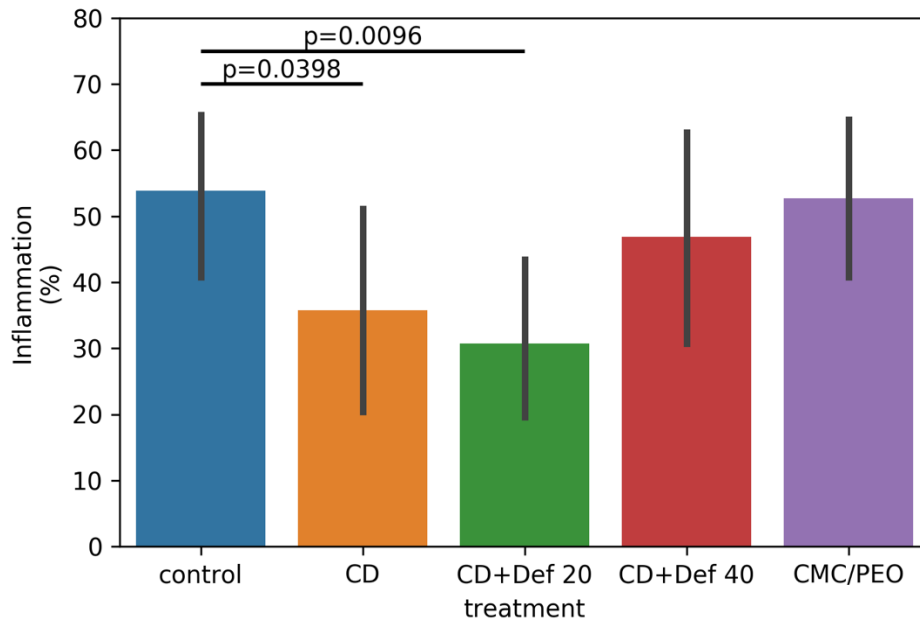


Fig. 4. Histological grading of inflammation in the adhesion and surrounding area.

Foreign body-type chronic inflammation within the epidural scar was expressed as a percentage values. Graph showing the histological grading of inflammation in the post laminectomy site treated with Chitogel (CD), Chitogel with 20 mM Def (CD + Def20 mM), Chitogel with 40 mM Def (CD + 40 mM) and CMC/PEO in comparison to Control – Kaolin alone.

### Grade of inflammation

Control sites showed an inflammation grade of 53.8% [CI 36%, 69%]. There was evidence of foreign body (FB) type reaction characterised by dense infiltrates of predominantly late inflammatory cells, especially macrophages with engulfed FB material.

Application of Chitogel significantly reduced this inflammation by 18.1 % compared to Control, with a mean inflammation grade of 35.7% [CI 21%, 51%],  $p=0.0398$  (Fig 4). From all test treatments, Chitogel + Def 20mM appeared to have the strongest effect, reducing the inflammation by 23.1% compared to Control with a mean inflammation grade of 30.7% [CI 16%, 46%],  $p<0.0096$ . Whereas Chitogel + Def 40 mM and CMC/PEO (52.69% [CI 37.46, 67.91]) was unable to reduce inflammation (47% [CI 32%, 62%]) compared to no treatment control ( $p>0.05$ )).

### **Discussion:**

The current study shows Chitogel (a product of naturally occurring chitin) combined with Deferiprone 20 mM has significant anti-inflammatory properties and trends to reduce adhesions after laminectomy in comparison to no-treatment control and to commercially available CMC/PEO<sup>377,479</sup>. Even in the background of extensive inflammation and fibrosis caused by Kaolin, which promoted an environment for adhesion formation above and beyond what one would normally see in humans' post-surgery. Deferiprone-Chitogel could effectively reduce the inflammation and associated fibroblastic activity in the epidural region after surgery. All detected Def concentrations in plasma were substantially below the maximum Def plasma concentrations considered to be safe by the FDA<sup>480</sup>. This makes Def-Chitogel a promising agent to reduce the morbidity associated with Failed Back Surgery Syndrome.

Current strategies to prevent epidural fibrosis include modifications to surgical technique, radiation lysis, using medications and physical barrier materials<sup>387</sup>. Each of these techniques has its limitations, yet barrier devices appear to be the preferred option with several products in preclinical and clinical development. Even though this appears to be among the more promising techniques, an ideal barrier or scaffolding material is still elusive. Autologous fat

has been used as a physiological barrier for a long time with limited long-term benefit<sup>387</sup>. Natural polymers and synthetic polymers have been tried as combined barriers, where scaffolds are incorporated with drugs that could give an added benefit. The polymers that have been studied as biomaterials in the last decade are Cross-linked hyaluronic acid gel<sup>390</sup>, amniotic membrane<sup>481</sup> and silk-polythene glycol<sup>388</sup>. All these have a limited bioavailability and hence limited clinical use. Synthetic barriers are often combined with drugs such as Gelatine sponge with dexamethasone<sup>417</sup>, polyethylene glycol (PEG) with mitomycin-C, poly galacturonic acid gel (PGA) with Ibuprofen and Fibrin glue with methyl acrylate, but none have been recommended for standard practice. Scaffolds like Gelatine an inert spacer takes a long time to disintegrate and fibrin glue degrades faster than needed<sup>387</sup>. Pharmacological agents have had some limited benefit such as steroids, but some remain toxic to tissues like mitomycin, hence there is still an ongoing search for a better alternative<sup>473</sup>. Chitogel, a natural occurring biomaterial as an hydrogel barrier with its beneficial properties can be used as a scaffold to carry drugs that are safe and effective to regulate wound healing<sup>390</sup>.

Haemostasis being the first step in wound healing, optimal haemostasis is essential in promoting a balanced healing process. Chitogel is a rapid haemostatic agent when applied in the surgical bed and is thought to reduce scarring in part by promoting haemostasis<sup>44</sup>. Inflammation is the second step wherein the chemokines released from hematomas<sup>388</sup> lead to recruitment of neutrophils and macrophages, producing inflammatory cytokines and reactive oxygen species (ROS) followed by fibroblast migration and proliferation into the wound, which are critical factors in the third step of the healing process.

This fibroblastic activity in the extracellular space is regulated by various cytokines, such as transforming growth factor (TGF)-b1, interleukin-6 (IL-6) and fibroblast growth factor (FGF)<sup>387</sup>. Chitogel has previously been shown to have inherent anti-inflammatory effects and

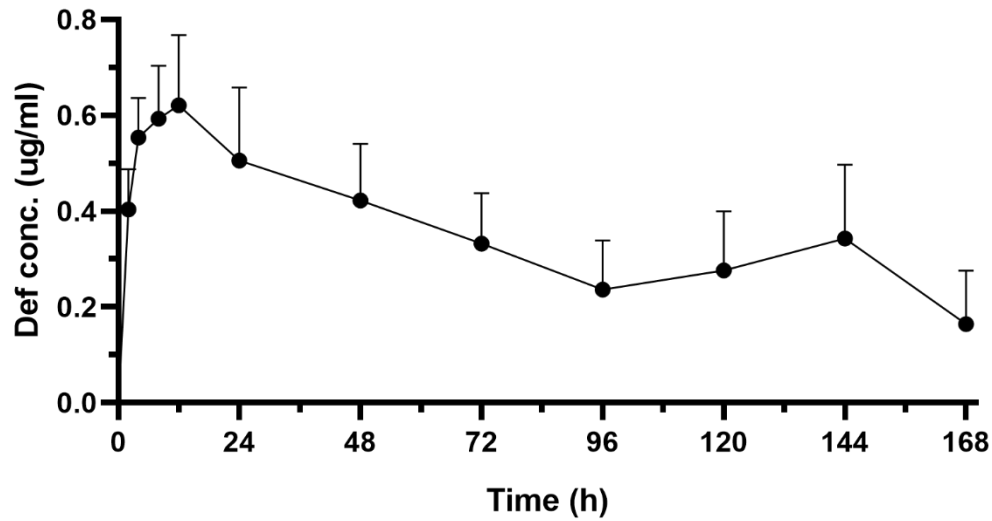
can reduce fibroblast migration *in vitro*, potentially contributing to its anti-adhesive properties<sup>80</sup>. These properties could be further enhanced by adding Deferiprone to the gel. Indeed, *in vitro* studies have shown potent anti-inflammatory and inhibitory effects on fibroblast migration, proliferation and collagen secretion with inhibition of IL-6, a proinflammatory cytokine<sup>85</sup>. Hence, the enhanced anti-inflammatory property of Chitogel +Def 20mM is noticed in comparison to Chitogel alone as evidenced in the present study, indicating Deferiprone exerts anti-inflammatory effects also *in vivo*. The peak concentration of plasma Deferiprone was seen at 12 hrs with Deferiprone levels further decreasing up to day 7 after which Deferiprone was barely detected anymore. Similarly, a gradual drug release from Chitogel was seen *in vitro* over 10 days with a maximum release reached after 24-48 hours. Importantly, the initial burst release of Deferiprone and continued release of Deferiprone in the first week after surgery matches the time frame of the wound healing process that is critically important to control at the second and third stages of wound healing<sup>85,365</sup>.

A previous 3-level sheep laminectomy study compared the anti-adhesive properties of Chitogel, Gelfoam and saline using MRI scan, “Peel test” and histopathology. Whilst the peel test showed reduced adhesions in the Chitogel treated sheep compared to control, there were no significant differences in adhesion scores on MRI scan or histopathology<sup>417</sup>. This current study also evaluated the nature of scar formed in the surgical bed using MRI images, “the gold standard” for evaluating FBSS<sup>473</sup>. Peel test was not used to keep the fibrosis intact for histology which showed extensive fibrosis associated with manifest inflammation induced by Kaolin. This was further confirmed using MRI where Kaolin control sites consistently showed evidence for adhesion formation and this may in part explain why statistical significance was not achieved in the Chitosan with Def groups in this study. Whilst Kaolin can thus be used to induce adhesions, providing a consistent positive control lesser dosage of

Kaolin might be preferable in future studies to elucidate the beneficial effects of the gel and its combination with Deferiprone. The histological evaluation showed that the scar formed in the gel treated groups had decreased density of collagen fibers as evidenced on Masson's trichrome staining. In spite of extensive foreign body reaction due to Kaolin, there was significant reduction in the inflammation in the Chitogel with 20 mM Deferiprone site, but the same was not seen with higher concentrations of Deferiprone. Dose dependent anti-inflammatory effects and dose-dependent effects on reducing fibroblast migration have been shown with dosages up to 20 mM<sup>14,85</sup>. This concentration seems to be ideal for wound healing, whereas higher dosage at 40 mM proved to be harmful, probably due to redox imbalance of ROS, a critical mechanism in injured tissues affecting the wound healing process<sup>482,483</sup>.

One of the limitations in this study was the 5-level laminectomy done for every sheep to increase the power and reduce the number of animals required. This resulted in the death of 2 sheep and post-mortem evaluation showed intrathecal bleeding which is not a rare complication, most often due to iatrogenic trauma to the spinal cord<sup>484</sup>. Our previous study used a 3-level laminectomy in the same animal species which in future studies will be our preferred model as it appeared to be better tolerated without major operative morbidity (14).

**Conclusion:** Chitogel with Deferiprone is a promising, composite immunomodulatory barrier agent possessing haemostatic and potent anti-inflammatory properties to prevent epidural adhesions and FBSS.



Supplementary Fig. 1. Safety profile of Serum Deferiprone. Deferiprone (Def) plasma concentrations ( $\mu\text{g/ml}$ ) over 7 days  $\pm$  SEM. The maximum Def concentration was reached after 12 hours ( $0.62 \mu\text{g/ml}$  Def) in the sheep treated with Chitogel-Def gel. After 7 days the Def plasma concentration decreased to  $0.16 \mu\text{g/ml}$  and after 3 weeks Def was not detected anymore.



## 9 SYNOPSIS OF THESIS

### Summary of Thesis:

Scar formation after wound healing has detrimental effects on surgical management of diseases in the human body. The endeavour of this research was to identify various contributors to scar formation and to find ways to alleviate this complication by the use of Chitogel in 3 different systems: 1. Nose - ENT surgery, 2. Abdomen in General Surgery and 3. Spine surgery after laminectomy. We aimed at studying the safety of Chitogel, a naturally derived polysaccharide that has properties of haemostasis and wound healing by inhibiting fibroblasts along with novel drugs -Deferiprone and Gallium Protoporphyrin, that are being repurposed from their original intended use in medical science. Deferiprone an Iron chelator that has been very successfully being used for the treatment of Thalassemia, a condition in which there is excess residual iron in the body due to abnormal red blood cells being broken down earlier than usual. Deferiprone has been tested in the lab and found to be safe and effective on human nasal epithelial cells in delaying the migration of fibroblasts which are essential component used by the body to build scar tissue. This along with Gallium – protoporphyrin which is a chemical with a similar structure to Haem in blood and has proven antibacterial effects invitro. Combining a hydrophilic haemostatic gel, a fibroblast migration inhibitor and a antibacterial drug is unique and therefore the subject of this thesis. Chitogel has also proved to be an excellent drug carrier and releases these drugs in an appropriate timeline which enables the drugs to produce an anti-inflammatory action along with an antibacterial effect and fibroblast inhibition. This thesis explored the use of the gel-drug combination in various surgical specialities, in various models and in various dosage combinations to find a safe and effective product that will be beneficial in each condition.

### **Chitogel with Def-GaPP in ENT-Endoscopic Sinus Surgery (ESS):**

Surgical outcomes in endoscopic sinus surgery are influenced by adequate haemostasis at the end of surgery and may be influenced by certain packing materials. Scar tissue formation in the region of the sinus opening may cause ostial stenosis leading to persistent inflammation and recalcitrant infections, needing revision surgery. In this study we aimed to determine the safety and efficacy of Chitogel with and without Deferiprone (Def) and Gallium Protoporphyrin <sup>89</sup> as adjuvants of wound healing and their ability to improve surgical outcome both subjectively and objectively in patients undergoing Full House FESS(FHF) and FHF with Drill out (DO). A Phase 1 randomised, blinded human clinical trial was conducted on consenting patients diagnosed with CRS and undergoing endoscopic sinus surgery. The Chitogel with and without a drug combination was instilled in the sinuses immediately after surgery on one side in FHF and the other side was considered as control in the same patients. In DO patients this was not possible hence a group of patients who did not receive the gel was considered as controls. Post operatively these patients were followed up at 2 weeks, 6 weeks and 12 weeks for evaluation of the validated quality of life Sino-Nasal Outcome Score (SNOT-22) and symptom based Visual Analogue score (VAS). The surgeon performing the endoscopy scored healing with the validated – Modified Lund Kennedy Score(LKS). Other parameters that were evaluated were: culture sensitivity pattern of sinus swabs to determine the presence or absence of *Staphylococcus Aureus* or *Pseudomonas Aeruginosa* at surgery and at 12 weeks, drug safety profile by testing inflammatory markers-Total blood cell counts, Liver function tests and Serum Ferritin levels at 0 hrs and 2 weeks after surgery, along with serum Deferiprone levels at 0 hrs, 2 hrs, 6 hrs and 2 weeks after surgery.

At the end of 12 weeks, among the patients who underwent DO there was a significant reduction in SNOT 22( $p<0.05$ ) and VAS significantly improved over a time period of 12 weeks in patient treated with Chitogel alone ( $p<0.05$ ). The ostium size of all the sinuses were measured and compared to 12 weeks PO, sinus ostial openings made during surgery and treated with Chitogel and Chitogel with Def and GaPP remained 90-100% the size of their original openings made at surgery. In comparison the DO patients who did not have any packing had shrinkage of the original ostium to 74% of the original ostial size. Similar trends were seen in Chitogel alone treated maxillary and sphenoid ostium in comparison to control as compared to baseline at surgery. Chitogel with Def treated frontal sinus ostia in the FHF groups were more ( $p<0.065$ ) patent as compared to their respective control (97% Vs 89%). The surgeon reported objective assessment with LKS score at 12 weeks, even though Chitogel treated sinuses appeared to heal better, there was no statistically significant difference in LKS for any of the test treatment groups when comparing the control untreated side with the test treatment side for each of those patient groups. Chitogel in combination with Def and GaPP as an antimicrobial did not alter the culture status significantly. Thus we were able to reiterate the fact that, Chitogel is an excellent post-operative dressing after ESS and has best patient reported symptom scores. Combination of Deferiprone and Gallium protoporphyrin though being safe, it had no positive beneficial effect on the ostium patency.

### **Chitogel with Def-GaPP in Abdominal Surgery:**

Adhesion formation after abdominal surgery is a common finding in the abdomen that leads to major and minor complications which include pain, obstruction and infertility. Even though there have been many anti-adhesive measures suggested none have been able to completely eliminate these abnormal scars. These complications come with an enormous

social and economic burden. In order to be able to evaluate this novel new treatment a suitable validated small animal model was developed. This study was a positive contribution as previously described models all lacked consistency in been able to produce a relatively standard adhesion. The new rat model of abdominal adhesions was done using Kaolin as the adhesion-inducing agent at an optimised dosage in 2 different abdominal surgery settings: 1. Intestinal Abrasion- mimicking a blunt injury with inflammation and 2. Abrasion + Enterotomy – a invasive procedure and infective model. In this model we evaluated the effect of various volumes of a Kaolin irritant, for adhesion formation as compared this to saline. The adhesion that formed were graded at 3 weeks and both macroscopically using pre-determined adhesion scores and microscopically on histopathology. We found that Kaolin at 0.005 g/mL caused consistent adhesions without compromising rat viability. Higher doses had significant morbidity and mortality. Using this newly proposed rat abdominal adhesion model we tested the anti-adhesive properties of Chitogel, Chitogel plus Deferiprone in varied dosages (5, 10 or 20 mM), together with Gallium Protoporphyrin (250µg/mL) as an anti-infection combination. The abrasion with enterotomy rats were randomised to receive saline, Chitogel or Chitogel with Deferiprone (1 or 5 mM). At the end point adhesions were graded macroscopically and microscopically; and also, the tensile strength of the scar on the repaired caecum was determined by an investigator blinded to the treatment groups.

We found that Chitogel with Deferiprone 5 mM significantly reduced adhesion formation ( $p < 0.01$ ) in the rat abrasion model. Chitogel with Deferiprone 5 mM and 1 mM also significantly reduced adhesions ( $p < 0.05$ ) after abrasion with enterotomy. Tensile strength testing of caecum specimens in the colon abrasion and enterotomy model showed Def-Chitogel treatment did not weaken the enterotomy site with Def 1mM Chitogel treated sites having significantly better tensile strength compared to control saline treated enterotomy rats. In conclusion, Chitogel with Deferiprone 1 mM constitutes an effective preventative anti-

adhesion barrier after abdominal surgery in a rat model. Moreover, this therapeutic combination of agents is safe and does not weaken the healing of the sutured enterotomy site.

### **Chitogel with Def in Spine Surgery:**

Failed Back Surgery Syndrome (FBSS) is increasingly occurring disease among the patients who have undergone spinal laminectomy - a common procedure performed to relieve neural compression in patients suffering from myelopathy or radiculopathy. Excessive scarring in the surgical bed is implicated as a cause and many strategies have been evaluated with success and there remains no FDA approved product registered in the USA for this indication. Hydrogels such as carboxy-methylcellulose and poly-ethylene-oxide (CMC-PEO) have been tried post laminectomy, however, their efficacy has also not been proven. We proposed to use Chitogel in combination with the iron chelator Deferiprone (Def) which has been shown to have fibroblast inhibitory effects in various dosages to attempt to prevent adhesion formation in a sheep laminectomy model.

Our research groups were Chitogel, Chitogel with Def at 20mM or 40mM or Carboxy-methylcellulose and Polyethylene oxide (CMC/PEO) gel. Post operatively the sheep were assessed at 3 months with Magnetic Resonance Imaging (MRI) and histology for the presence and extent of adhesions. MRI and histology assessment indicated that Kaolin induced severe inflammation with adhesion formation. Chitogel with and without 20 mM Def decreased inflammation ( $p<0.01$ ) and trended to reduce adhesions ( $p<0.1$ ). Chitogel with Def 40mM was similar to CMC/PEO and did not reduce inflammation or adhesions compared to no-treatment control. Once again, we found that Chitogel in combination with Def 20 mM is safe and effective in decreasing the inflammatory process and subsequent post-operative adhesions following laminectomy.

## **CONCLUSION:**

This doctoral thesis aimed to assess novel possible solutions for commonly occurring undesired effects of surgical treatment, i.e., scarring in diverse systems in the human body. We proposed to test the safety and efficacy of Chitogel incorporating Deferiprone and Gallium Protoporphyrin which enhance Chitogels' healing and anti-bacterial properties.

We are able to confidently conclude that Chitogel is an excellent hydrogel which not only promotes haemostasis and wound healing, but it acts as a drug carrier that is able to prevent ostial stenosis in the sinus cavity after sinus surgery. The patient reported outcomes were also significantly better than the no treatment control patients. One of the drawbacks of this trial was the number of patients we were able to test the gel-drug combination. Now that we know Chitogel with Def it is safe and there is not enough evidence for the combination with GaPP, a future study where Deferiprone in a lower dosage would be the ideal way forward.

In the abdomen we found that 1% Chitogel with Deferiprone was an ideal barrier agent that was able to be instilled in the abdominal cavity and that this prevented adhesion formation as seen in a standardized rat model. The resultant scar tissue produced in the presence of gel and Deferiprone did not weaken the bowel anastomosis as seen in the stretch test. Furthermore, a large animal model and a future human clinical trial are necessary to support the use of Chitogel with or without Deferiprone in Surgical practice .

Similarly, the anti-adhesive effect of Chitogel with 20 Mm of Deferiprone on a Kaolin induced scar over the dura of the spinal cord was not very pronounced, hence further small

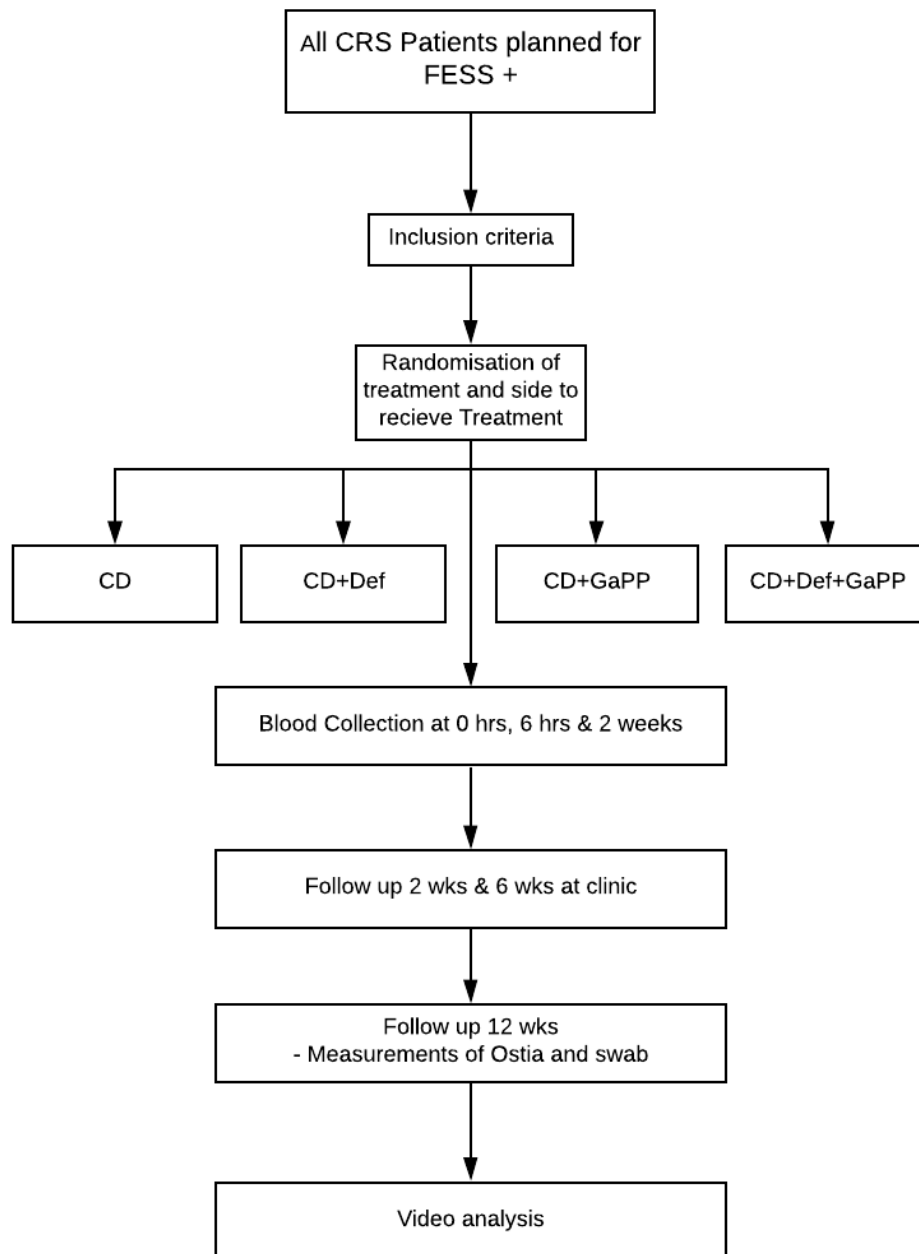
and large animal models are necessary to be done using Chitogel with a lower dosage of Def to fine tune the benefits.

These findings certainly helped us to understand the nature of scar formation and the role of Chitogel in preventing the debilitating effects of scar. We hope that this body of information will help surgeons and researchers to envisage a scar free surgery in the future.

## 10 Appendix

### Appendix 1: Flow chart of Project 1 Clinical trial

Recruitment of patients in Clinical trial and randomization





## Appendix 2: Consent and proforma

### Patient recruitment Checklist

(to be completed by surgeon at Time = 0)

Patient Name:

Date:

Clinician Name:

Inclusion criteria (Need to answer YES to all of these)

|                                                                                                        |                          |    |
|--------------------------------------------------------------------------------------------------------|--------------------------|----|
| Is the patient over 18 and able to give competent informed consent?                                    | Yes                      | No |
| Is the patient willing to return to the clinic at 2 weeks, 6 weeks and 12 weeks after recruitment?     | Yes                      | No |
| Has the patient had a previous sinus operation?<br>(More than 12 weeks ago)                            | Yes<br>Year(s):<br>_____ | No |
| Does the patient have symptoms of CRS?                                                                 | Yes                      | No |
| Does the patient have symptoms of active infection?                                                    | Yes                      | No |
| Does the patient have signs of CRS?                                                                    | Yes                      | No |
| Does the patient have signs of active infection?                                                       | Yes                      | No |
| Has the patient failed oral antibiotics therapy at least once prior to their recruitment to the study? | Yes                      | No |
| Have you taken a swab and sent it for MC&S?                                                            | Yes                      | No |

Exclusion criteria (Need to answer NO to all of these)

Chitogel Grading Pro forma – to be completed by surgeon

Date:

Patient Name:

Time of assessment: 0 week (at recruitment)/2 week/6 weeks / 12 weeks



Government of South Australia  
SA Health



**CENTRAL ADELAIDE LOCAL HEALTH NETWORK  
The Queen Elizabeth Hospital**

**Participant Information Sheet/Consent**

**Title:** Chitosan-dextran (Chitodex) gel with and without Deferiprone and Gallium-Protoporphyrin wound healing and post-operative outcomes in Chronic rhino-sinusitis.

HREC number: *HREC/17/TQEH/245*

CAHLN REF No: Q20171012

Protocol number: Version 2.5, 17/04/2018

Principal Investigator: Prof. P J Wormald

Associate Investigator: Dr Alkis Psaltis / Dr Sarah Vreugde / Dr Rajan Sundaresan V

Location of the Investigation: TQEH

---

**1. Invitation to Participate**

We invite you to take part in this research project because you have chronic rhinosinusitis and are about to undergo surgery. However, before you decide whether or not you want to participate, we need to be sure that you understand **Why we are doing it**, and **What would it mean if you agreed**.

This research project is testing a new treatment for chronic rhinosinusitis by the use of a Chitosan-Dextran (Chitodex) gel mixed with medications to see if there is improved healing after sinus surgery and less infection.

Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or your local doctor. You do not have to make an immediate decision, and should you agree to take part in the research project you are free to change your mind and withdraw at any stage of the trial without any effect on your relationship with your treating doctors or the hospital.

**2. Participation is Voluntary**

Participation in this research is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether or not you take part and will not affect your waiting list position for sinus surgery.

If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that, you:

- Understand what you have read
- Consent to take part in the research project
- Consent to have the tests and treatments that are described
- Consent to the use of your personal and health information as described.

You will be given a copy of this Participant Information and Consent Form to keep.

### 3. What is the purpose of this research?

**Back ground:** Chronic rhinosinusitis affects approximately 15% of the general population and is characterised by sinusitis symptoms persisting for more than 3 months. Patients who do not respond adequately to oral and topical steroids, antibiotics and nasal lavage require surgical management. The surgical procedure is termed endoscopic sinus surgery, and involves removing oedematous mucosa, pus and debris, as well as clearance of bony walls within the sinonasal cavity to open up blocked sinuses. You will receive the standard hospital information sheet about the sinus surgery, and you will sign the standard hospital consent form for the sinus surgery.

**Purpose:** The purpose of this research project is testing in sinus surgery whether adding two new antimicrobial agents (Deferiprone and Gallium-Protoporphyrin) to a locally developed dissolvable nasal dressing (Chitodex gel) will improve the anti-microbial and wound-healing effects of Chitodex gel after sinus surgery compared to chitodex alone.

Why is the research being done?

The research is being done to improve the results from sinus surgery by: improving the healing process in the nasal/sinus cavities; reducing infection; and reducing the need for revision sinus surgery.

### 4. Who is sponsoring the trial and is there any benefits money being paid to the researcher/ Department/anyone else.

The Department of ENT and University of Adelaide is funding the research and there are no payments being done to the research or the department or participants or any company. Professor Peter-John Wormald, the Principal Investigator of this study is part of a consortium that owns the patent for Chitodex gel which is being used in this study. Professor Wormald is a cofounder of Chitodex and has been involved in the development of this product since its inception. He currently co-owns the patent for use of Chitodex in sinus surgery. However, he will not play any role in the data analyses since it is being done by the members of the ENT Department who do not have any financial interest in the outcome of this study.

### 5. How and why have I been chosen as a possible participant in the research?

You have been invited because you have been diagnosed to have chronic sinusitis and will be undergoing endoscopic sinus surgery (ESS). You will be randomly (like the toss of a coin) allocated to receive one of the three treatments to one side of the sinus surgery (ie left or right) or if its a frontal drill out into a fourth group (control) receiving saline and the results compared to see if one treatment is more effective. .

### 6. How many other people have been asked to consider participating?

A total of 90 participants will be involved in this trial.

### 7. What does participation in this research involve?

**Procedure and Treatment:** Under endoscopic guidance, each participant will have a sinus swab performed prior to surgery and then undergo the planned sinus surgery-Endoscopic sinus surgery (ESS) or ESS with frontal drill out (explained and consented with separate forms).

1. At the end of the procedure, each participant will receive 10 ml of gel (Chitodex or Chitodex+Deferiprone or Chitodex+Gallium-Protoporphyrin or Chitodex+ Deferiprone+ Gallium-Protoporphyrin) into one side of each of the three sinuses and the non-treated side would be referred to as the control and receive routine standard of care.
2. If the surgery is meant to produce a larger frontal sinus cavity (ESS with frontal drillout) 20 ml of gel would be applied or 20 ml of saline if you are in the control group.
3. Post-operative care will proceed as per standard care after sinus surgery (except for blood tests and questionnaire as described below).

4. You will return to the outpatient department 2 weeks, 6 weeks and 12 weeks after the surgery for post-operative review. During each visit we would perform a sinus swab and an endoscopic video recording of your sinuses.

The recorded video examination will then be scored by an independent clinician, unaware of your treatment, for infection (pus), edema, granulation tissue, and crusting using a standardised scoring scales.

**8. What would I be asked to do at each visit?**

1. You will be asked to complete a self-directed symptom and comfort questionnaire at each review which is specific for each side.
2. Routine blood tests (haematology with neutrophil counts and blood chemistry with liver enzymes) will occur at day 0 and at first post-operative visit at 2 weeks. Deferiprone and Gallium-Protoporphyrin serum concentrations will also be measured at the same time and also at 2 hrs and 6 hrs after surgery.

**9. What if my problems persist or I don't feel relief?**

If there are signs of persistent infection at any visit, then a second swab will be taken and sent for repeat microbiological evaluation and you will receive antibiotics as directed by its report. If you have not improved, you will resume usual outpatient/surgical care for your symptoms. If there are no further clinical signs of infection at the 12-week post-surgery visit, you will have a final microbiology swab taken to confirm eradication of infection and would be considered as having completed the study. The research project has been designed to make sure the researchers interpret the results in a fair and appropriate way and avoids study doctors or participants jumping to conclusions.

**10. Does my participation involve extra cost?**

There are no additional costs associated with participating in this research project, nor will you be paid. All medication, tests and medical care required as part of the research project will be provided to you free of charge. Parking is available at the TQEH which is free for first 2 hrs and arrangements for longer stay will be done by the Department (discuss with the study doctor). Your local doctor will be advised of your decision to participate in this research project.

**11. Do I have any restrictions while participating in the trial?**

Participating in this study does not imply any restrictions in lifestyle, physical activity, diet or medication use, aside from that for the usual post-operative care for sinus surgery. There is no restriction of diet and can take all regular medication. You cannot participate in this study if you have allergy to shell fish and any specific drug allergy to the drugs being tested or have a history of being diagnosed with any type of hepatitis in the past or during the preparative evaluation.

**12. Other relevant information about the research project**

The results of this research will be used by the study doctor Dr. Rajan Sundaresan VEDIAPPAN to obtain a Doctorate in Philosophy (Ph.D.)

**13. What are the alternatives to participation?**

Participation in this trial is not compulsory to receive treatment at this hospital. Other options are available; these include oral antibiotics with or without surgery & long term steroidal nasal spray. Your study doctor will discuss these options with you before you decide whether or not to take part in this research project. You can also discuss the options with your local doctor.

#### 14. What are the possible benefits of taking part?

We cannot guarantee or promise that you will receive any benefits from this research; however, possible benefits may include better healing and decrease in the recurrence of infection.

#### 15. What treatments are used in the research project?

The research project involves use of a Gel called Chitodex (CD) containing Chitosan derived from crustaceans and dextran with a new antimicrobial drug combination.

Test Group 1: receives 10ml of CD gel in one side of the sinus after surgery

Test Group 2: receives 10 ml of CD gel with Deferiprone (Def) in one side of the sinus after surgery

Test Group 3: receives 10 ml of CD gel with Gallium-Protoporphyrin (GaPP) in one side of the sinus after surgery

Test Group 4: receives 10 ml of CD gel with Deferiprone and Gallium-Protoporphyrin (GaPP) in one side of the sinus after surgery

Test Group 5(only in ESS with Frontal drill out): 20 ml of saline

In participants having a larger sinus cavity after surgery (ESS + frontal drill out)-double the usual dosage ie 20 ml of gel, will be applied. Chitosan the main ingredient in Chitodex gel is already being used in numerous applications for its preservation function (in foods, agriculture, cosmetics and toothpastes), for its antibacterial function (as a coating to fruits and vegetables), for its hydrating properties (in cosmetics) . Medically, it is well recognised for its haemostatic properties (e.g. US Military as a haemostatic dressing), anti-adhesive and pro-wound healing properties, as well as for its antimicrobial actions. At the TQEH we have been using this gel in the past few years in clinical trials and have found to have both anti adhesive properties and also helps in achieving haemostasis. This is currently approved by the FDA for use in treatment of sinusitis, but not approved by the TGA for use in Australia.

Deferiprone is an iron chelator-which means it removes excess iron from the blood in patients with a blood disorder called Thalassaemia major and also has properties that could treat infection. Deferiprone as an oral formulation is TGA approved for the treatment of iron overload in thalassaemia major: GaPP, Chitodex gel and Deferiprone have not been approved by the TGA for topical application.

#### 16. What are the possible risks and disadvantages of taking part?

##### General:

Medical treatments often cause side effects. You may have none, some or all of the effects listed below, and they may be mild, moderate or severe. If you have any of these side effects, or are worried about them, talk with your study doctor. Your study doctor will also be looking out for side effects.

There may be side effects that the researchers do not expect or do not know about and that may be serious. Tell your study doctor immediately about any new or unusual symptoms that you get.

Many side effects go away shortly after treatment ends. However, sometimes side effects can be serious, long lasting or permanent. If a severe side effect or reaction occurs, your study doctor may need to stop your treatment. Your study doctor will discuss the best way of managing any side effects with you. The general drug allergy symptoms to watch for would be skin rashes, itching, burning sensation in throat and stomach, vomiting or bleeding from the nose.

The only possible side effect would be allergy to Chitosan for individuals who have allergy to shrimps or other seafood. In all trials that have been conducted in this department involving Chitodex as a topical product in human subjects, there have been no adverse effects.

Deferiprone orally in large doses may cause decrease in white blood cells but this is unlikely to occur with the very low doses in this research project. You will be monitored by the team regularly. The doses of Deferiprone from the topical application are much times lower than the dose approved to treat iron overload in patients. The reported common side effects of colored urine, joint pains and aches, nausea, vomiting, abdominal pain and lowering the white cell count in the blood for the tablet form of Deferiprone are extremely unlikely to occur with the topical Deferiprone in the gels being used in this study

Gallium and gallium salts are safe and widely used in a range of biomedical applications including as (radio) pharmaceuticals.

We therefore expect no local or systemic toxicity issues from the topical application of the Def-GaPP-CD-gel in this research project.

**Pregnancy & Child bearing:**

The effects of Chitosan gel and the drugs Deferiprone and Gallium-Protoporphyrin on the unborn child and on the newborn baby are not known. Because of this, it is important that research project participants are not pregnant or breast-feeding and do not become pregnant during the course of the research project. You must not participate in the research if you are pregnant or trying to become pregnant, or breast-feeding.

All participants with the capacity to achieve a pregnancy ie women of child bearing potential or men with partners having child bearing potential must practice highly effective contraception one month prior to and during the study. Discuss with the study doctor

**17. What will happen to my test samples?**

Samples (blood and swabs) will be collected and stored safely in SA pathology/Clipath facility for evaluation of liver function test, routine blood analysis for markers of chronic infection once before surgery and 2 weeks post-surgery. Separate samples will be collected at these timings and also at 2 hrs and 6hrs post-surgery for evaluation of Deferiprone and GaPP levels in the serum. Once analysed, samples will be disposed of according to SA Pathology protocol by the end of the study.

**18. What if new information arises during this research project?**

Sometimes during the course of a research project, new information becomes available about the treatment that is being studied. If this happens, your study doctor will tell you about it and discuss with you whether you want to continue in the research project. If you decide to withdraw, your study doctor will make arrangements for your regular health care to continue. If you decide to continue in the research project you will be asked to sign an updated consent form.

Also, on receiving new information, your study doctor might consider it to be in your best interests to withdraw you from the research project. If this happens, he/ she will explain the reasons and arrange for your regular health care to continue.

**19. Can I have other treatments during this research project?**

It is important to tell your study doctor and the study staff about any treatments or medications you may be taking, including over-the-counter medications, vitamins or herbal remedies, acupuncture or other alternative treatments. You should also tell your study doctor about any changes to these during your participation in the research project.

## **20. What if I withdraw from this research project?**

If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to discuss any special requirements linked to withdrawing.

If you do withdraw your consent during the research project, the study doctor and relevant study staff will not collect additional personal information from you, although information already collected will be retained to ensure that the results of the research project can be measured properly and to comply with law. You should be aware that data collected up to the time you withdraw will form part of the research project results. If you do not want them to do this, you must tell them when you withdraw.

## **21. Could this research project be stopped unexpectedly?**

This research project may be stopped unexpectedly for a variety of reasons. These may include reasons such as:

- Unacceptable side effects
- The drug/treatment/device being shown not to be effective
- The drug/treatment/device being shown to work and not need further testing

## **22. What happens when the research project ends**

You will receive standard care in the form of regular follow ups and routine blood tests in case of any more infections or anaemia.

## **23. What will happen to information about me?**

By signing the consent form, you consent to the study doctor and relevant research staff collecting and using information about you for the research project. Any information obtained in connection with this research project that can identify you (eg: name, DOB, contact details) will remain confidential and be kept linked to your study code in a separate, securely stored file accessible to the investigators only. All information collected in the research project will be re-identifiable by the investigators only, and will be stored securely in locked cupboards hard copy and on password protected computers in the Department of Otorhinolaryngology which is out of bounds for unauthorised staff or public. Your information will only be used for the purpose of this research project and it will only be disclosed with your permission, except as required by law.

Information about you may be obtained from your health records held at this and other health services for the purpose of this research. By signing the consent form you agree to the study team accessing health records if they are relevant to your participation in this research project.

Your health records and any information obtained during the research project are subject to inspection (for the purpose of verifying the procedures and the data) by the relevant authorities of Therapeutic Goods Administration or CAHLN, or as required by law. By signing the Consent Form, you authorise release of, or access to, this confidential information to the relevant study personnel and relevant authorities as noted above.

## **24. What would be done with the information gained regarding this study?**

It is anticipated that the results of this research project will be published and/or presented in a variety of forums. In any publication and/or presentation, information will be provided in such a way that you cannot be identified, except with your permission. In no instance except as demanded by law will the name of an individual be revealed.

**25. What if I have any injuries during the research project?**

If you suffer any injuries or complications as a result of this research project, you should contact the study team as soon as possible and you will be assisted with arranging appropriate medical treatment. If you are eligible for Medicare, you can receive any medical treatment required to treat the injury or complication, free of charge, as a public patient in any Australian public hospital. Your participation in this study does not affect any other right you may have to compensation under common law.

**26. Who is organising and funding the research is there any financial benefit?**

This research project is being conducted by Professor Peter-John Wormald and is the co-patent holder for the products used and also for the novel therapy.

You will not benefit financially from your involvement in this research project even if, for example, your blood samples (or knowledge acquired from analysis of your samples) prove to be of commercial value to The University of Adelaide.

In addition, if knowledge acquired through this research leads to discoveries that are of commercial value to The University of Adelaide, the study doctors or their institutions, there will be no financial benefit to you or your family from these discoveries.

No member of the research team will receive a personal financial benefit (except potential benefit from patent held by the primary investigator) from your involvement in this research project (other than their ordinary wages).

**27. Who has reviewed the research project?**

All research in Australia involving humans is reviewed by an independent group of people called a Human Research Ethics Committee (HREC). The ethical aspects of this research project have been approved by the Human Research Ethics Committee (TQEH/LMH/MH). This project will be carried out according to the *National Statement on Ethical Conduct in Human Research (2007)*. This statement has been developed to protect the interests of people who agree to participate in human research studies.



## 28. Further information and who to contact

The person you may need to contact will depend on the nature of your query.

If you want any further information concerning this project or if you have any medical problems which may be related to your involvement in the project (for example, any side effects), you can contact the assistant investigator at TQEH on 0423-674912 or any of the following people:

|           |                                                                                                |
|-----------|------------------------------------------------------------------------------------------------|
| Name      | Bernadette Swart                                                                               |
| Position  | CALHN Research Manager                                                                         |
| Telephone | <b>(08) 7117 2229</b> (Roma Mitchell House), (08) 8222 6841 (TQEH)                             |
| Email     | <a href="mailto:health.CALHNResearchEthics@sa.gov.au">health.CALHNResearchEthics@sa.gov.au</a> |

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about being a research participant in general, then you may contact:

|                        |                                                                                                |
|------------------------|------------------------------------------------------------------------------------------------|
| Reviewing HREC name    | Human Research Ethics Committee (TQEH/LMH/LH)                                                  |
| HREC Executive Officer | Ms Heather O'Dea                                                                               |
| Telephone              | <b>(08) 7117 2229</b> (Roma Mitchell House), (08) 8222 6841 (TQEH)                             |
| Email                  | <a href="mailto:health.CALHNResearchEthics@sa.gov.au">health.CALHNResearchEthics@sa.gov.au</a> |



Government of South Australia  
SA Health



**CENTRAL ADELAIDE LOCAL HEALTH NETWORK  
The Queen Elizabeth Hospital**

**CONSENT FORM**

**Title** Chitosan-dextran (Chitodex) gel with and without Deferiprone and Gallium-Protoporphyrin wound healing and post-operative outcomes in Chronic rhino-sinusitis.

**Declaration by Participant**

I have read the Participant Information Sheet, or someone has read it to me in a language that I understand. The research worker has explained to me all the aspects of this study and I voluntarily consent to participate.

I understand the purposes, procedures and risks of the research described in the Participant Information Sheet.

I give permission for my doctors, other health professionals, hospitals or laboratories outside this hospital to release information to The Queen Elizabeth Hospital/The Memorial Hospital concerning my disease and treatment for the purposes of this project. I understand that such information will remain confidential.

I have had an opportunity to ask questions and I am satisfied with the answers I have received.

I freely agree to participate in this research project as described and understand that I am free to withdraw at any time during the study without affecting my future health care.

I have been explained in the presence of my family member that my identity will be kept confidential and permit if any information has to be shared with my general practitioner.

I understand that I will be given a signed copy of this document to keep.

I the undersigned hereby consent to my involvement in the research project explained above.

Name of Participant (please print)

Signature:

Date:

**Declaration by Study Doctor/Senior Researcher<sup>†</sup>**

I have given a verbal explanation of the research project, its procedures and risks and I believe that the participant has understood that explanation.

Name of Study Doctor/Senior Researcher

Signature

Date

|                                                |                                                                       |                                |                                                                       |          |
|------------------------------------------------|-----------------------------------------------------------------------|--------------------------------|-----------------------------------------------------------------------|----------|
|                                                | <u>L</u>                                                              | <u>R</u>                       |                                                                       |          |
| <u>ADHESIONS</u>                               |                                                                       |                                |                                                                       |          |
| <u>% Middle Turbinate taken up by adhesion</u> | <u>0, 1-25, 26-50, &gt;50%</u>                                        | <u>0, 1-25, 26-50, &gt;50%</u> |                                                                       |          |
| <u>Adhesions Divided</u>                       | <u>Yes/No</u>                                                         | <u>Yes/No</u>                  |                                                                       |          |
| <u>EVIDENCE OF INFECTION (PUS)</u>             |                                                                       |                                |                                                                       |          |
| <u>Ordinal Scale (0-2)</u>                     | <u>No visible evidence of infection</u>                               | <u>0</u>                       | <u>No visible evidence of infection</u>                               | <u>0</u> |
|                                                | <u>Mild mucopurulent drainage</u>                                     | <u>1</u>                       | <u>Mild mucopurulent drainage</u>                                     | <u>1</u> |
|                                                | <u>Gross mucopurulent drainage with obvious frank infection</u>       | <u>2</u>                       | <u>Gross mucopurulent drainage with obvious frank infection</u>       | <u>2</u> |
| <u>Pus swab taken</u>                          | <u>Yes/No</u>                                                         | <u>Yes/No</u>                  |                                                                       |          |
| <u>MUCOSAL OEDEMA</u>                          |                                                                       |                                |                                                                       |          |
| <u>Ordinal scale (0-3)</u>                     | <u>No visible mucosal oedema</u>                                      | <u>0</u>                       | <u>No visible mucosal oedema</u>                                      | <u>0</u> |
|                                                | <u>Mild mucosal oedema without obliteration of the ethmoid cavity</u> | <u>1</u>                       | <u>Mild mucosal oedema without obliteration of the ethmoid cavity</u> | <u>1</u> |
|                                                | <u>Severe mucosal oedema obliterating most of the ethmoid cavity</u>  | <u>2</u>                       | <u>Severe mucosal oedema obliterating most of the ethmoid cavity</u>  | <u>2</u> |
|                                                | <u>Frank polyposis</u>                                                | <u>3</u>                       | <u>Frank polyposis</u>                                                | <u>3</u> |
| <u>CRUSTING</u>                                |                                                                       |                                |                                                                       |          |
| <u>Ordinal Scale (0-2)</u>                     | <u>Absent</u>                                                         | <u>0</u>                       | <u>Absent</u>                                                         | <u>0</u> |
|                                                | <u>Mild</u>                                                           | <u>1</u>                       | <u>Mild</u>                                                           | <u>1</u> |
|                                                | <u>Severe</u>                                                         | <u>2</u>                       | <u>Severe</u>                                                         | <u>2</u> |
| <u>Debridement</u>                             | <u>Yes/No</u>                                                         | <u>Yes/No</u>                  |                                                                       |          |
| <u>GRANULATIONS</u>                            |                                                                       |                                |                                                                       |          |
| <u>Ordinal Scale (0-3)</u>                     | <u>No visible granulations</u>                                        | <u>0</u>                       | <u>No visible granulations</u>                                        | <u>0</u> |
|                                                | <u>Mild</u>                                                           | <u>1</u>                       | <u>Mild</u>                                                           | <u>1</u> |
|                                                | <u>Moderate</u>                                                       | <u>2</u>                       | <u>Moderate</u>                                                       | <u>2</u> |
|                                                | <u>Severe</u>                                                         | <u>3</u>                       | <u>Severe</u>                                                         | <u>3</u> |

| <u>OSTIAL MEASUREMENTS</u><br>(in mm <sup>2</sup> ) | <u>R</u>                        |  | <u>L</u>                        |  |
|-----------------------------------------------------|---------------------------------|--|---------------------------------|--|
| <u>Frontal</u>                                      |                                 |  |                                 |  |
| <u>Maxillary</u>                                    |                                 |  |                                 |  |
| <u>Ethmoid</u>                                      |                                 |  |                                 |  |
| <u>Sphenoid</u>                                     |                                 |  |                                 |  |
| <u>Nasal Swab</u>                                   | <u>Infection present/absent</u> |  | <u>Infection present/absent</u> |  |
|                                                     |                                 |  |                                 |  |
|                                                     |                                 |  |                                 |  |

## Appendix 3: Patient reported Objective score sheet

Symptom questionnaire



**Government of South Australia**  
**Central Northern Adelaide**  
**Health Service**

CENTRAL ADELAIDE LOCAL HEALTH NETWORK

The Queen Elizabeth Hospital & Lyell McEwin Hospital

SELF-DIRECTED SYMPTOM AND COMFORT form

Title: Chitosan-dextran (Chitogel) with and without Deferiprone and Gallium-Protoporphyrin: wound healing and post-operative outcomes in Chronic rhinosinusitis.

Protocol Number:

Dear Participant,

Thank you for your participation in this study.

The following evaluation form should only take you less than 5 minutes to complete.

The purpose of this evaluation form is to assess symptom outcomes from your point of view as the patient, which is an important consideration in the outcomes of this study.

On the page attached, please indicate your assessment of the following factors on the scale provided (0-10)

Yours sincerely,

Assistant Researcher

Name: \_\_\_\_\_

Today's Date: \_\_\_\_\_

Time of assessment: At recruitment, Time = 0 weeks

2 weeks / 6 weeks/12 weeks since recruitment

Explanations of scale:

Facial Pain/Discomfort: 0 is no pain or discomfort, 10 is the worst pain you have ever experienced

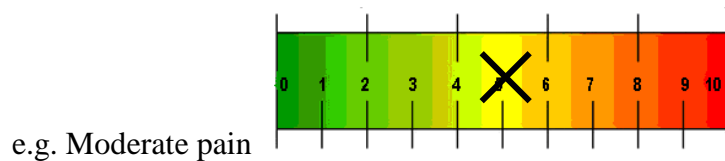
Bleeding: 0 is no bleeding, 10 is bleeding requiring re-operation to control it

Nasal Obstruction: 0 is a perfect airway which is very easy to breathe through, 10 is completely blocked with no air movement through that side.

Nasal Secretion/throat drip: 0 is no nasal secretions, 10 is copious secretions with constant nasal dripping.

Throat drip: 0 is no nasal secretions, 10 is copious secretions with constant nasal dripping.

Sense of smell: 0 is no smell ability at all, 10 is best smell ability.



Please evaluate left and right sides separately

|                                    | Left | Right |
|------------------------------------|------|-------|
| Facial<br>pain/<br>discomfort      |      |       |
| Bleeding –<br>Nose and<br>Throat   |      |       |
| Nasal<br>Obstruction/<br>Breathing |      |       |
| Nasal<br>secretions                |      |       |
| Drip at<br>back of<br>throat       |      |       |
| Sense of<br>smell                  |      |       |





## 11 Bibliography:

1. [https://www.blueletterbible.org/kjv/jer/30/1/s\\_775001](https://www.blueletterbible.org/kjv/jer/30/1/s_775001) JKRf.
2. Bhattacharya S. Wound healing through the ages. *Indian J Plast Surg.* 2012;45(2):177-179.
3. Wohlfahrt T, Rauber S, Uebe S, et al. PU.1 controls fibroblast polarization and tissue fibrosis. *Nature.* 2019;566(7744):344-349.
4. Ahmed S, Ikram S. Chitosan Based Scaffolds and Their Applications in Wound Healing. *Achievements in the Life Sciences.* 2016;10(1):27-37.
5. Broughton G, 2nd, Janis JE, Attinger CE. A brief history of wound care. *Plast Reconstr Surg.* 2006;117(7 Suppl):6S-11S.
6. Majno G. *The healing hand : man and wound in the ancient world.* Cambridge, Mass: Harvard University Press; 1975.
7. Cohen IK. Lessons from the history of wound healing. *Clin Dermatol.* 2007;25(1):3-8.
8. Helling TS, McNabney WK. The role of amputation in the management of battlefield casualties: a history of two millennia. *J Trauma.* 2000;49(5):930-939.
9. Caldwell MD. Wound surgery. *Surg Clin North Am.* 2010;90(6):1125-1132.
10. Teller P, White TK. The physiology of wound healing: injury through maturation. *Surg Clin North Am.* 2009;89(3):599-610.
11. <http://www.merriam-webster.com> M-WD.
12. Capper D, Engel NW, Stichel D, et al. DNA methylation-based reclassification of olfactory neuroblastoma. *Acta Neuropathol.* 2018;136(2):255-271.
13. Waller DG, Sampson AP. 11 - Haemostasis. In: Waller DG, Sampson AP, eds. *Medical Pharmacology and Therapeutics (Fifth Edition).* Elsevier; 2018:175-190.
14. Mohammadpour M, Behjati M, Sadeghi A, Fassihi A. Wound healing by topical application of antioxidant iron chelators: kojic acid and deferiprone. *Int Wound J.* 2013;10(3):260-264.
15. Romagnani P, Lasagni L, Annunziato F, Serio M, Romagnani S. CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends in Immunology.* 2004;25(4):201-209.
16. Qing C. The molecular biology in wound healing & non-healing wound. *Chin J Traumatol.* 2017;20(4):189-193.

17. Oncel M, Kurt N, Remzi FH, et al. The effectiveness of systemic antibiotics in preventing postoperative, intraabdominal adhesions in an animal model. *Journal of Surgical Research*. 2001;101(1):52-55.
18. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature*. 2008;453(7193):314-321.
19. Guo S, DiPietro LA. Factors affecting wound healing. *J Dent Res*. 2010;89(3):219-229.
20. Rowe F, Wormald R, Cable R, et al. The Sight Loss and Vision Priority Setting Partnership (SLV-PSP): overview and results of the research prioritisation survey process. *BMJ Open*. 2014;4(7):e004905.
21. Martin P, Leibovich SJ. Inflammatory cells during wound repair: the good, the bad and the ugly. *Trends Cell Biol*. 2005;15(11):599-607.
22. Dovi JV, Szpaderska AM, DiPietro LA. Neutrophil function in the healing wound: adding insult to injury? *Thromb Haemost*. 2004;92(2):275-280.
23. Zeng Q, Yu Z, You J, Zhang Q. Efficacy and safety of Seprafilm for preventing postoperative abdominal adhesion: systematic review and meta-analysis. *World J Surg*. 2007;31(11):2125-2131; discussion 2132.
24. ten Broek RP, Issa Y, van Santbrink EJ, et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. *BMJ*. 2013;347:f5588.
25. Munireddy S, Kavalukas SL, Barbul A. Intra-abdominal healing: gastrointestinal tract and adhesions. *Surg Clin North Am*. 2010;90(6):1227-1236.
26. Ross JS, Robertson JT, Frederickson RC, et al. Association between peridural scar and recurrent radicular pain after lumbar discectomy: magnetic resonance evaluation. ADCON-L European Study Group. *Neurosurgery*. 1996;38(4):855-861; discussion 861-853.
27. LaRocca H, Macnab I. The laminectomy membrane. Studies in its evolution, characteristics, effects and prophylaxis in dogs. *J Bone Joint Surg Br*. 1974;56B(3):545-550.
28. Davey AK, Maher PJ. Surgical adhesions: a timely update, a great challenge for the future. *J Minim Invasive Gynecol*. 2007;14(1):15-22.
29. Valentine R, Wormald PJ. Nasal dressings after endoscopic sinus surgery: what and why? *Current opinion in otolaryngology & head and neck surgery*. 2010;18(1):44-48.
30. Morrissey DK, Bassiouni A, Psaltis AJ, Naidoo Y, Wormald PJ. Outcomes of revision endoscopic modified Lothrop procedure. *Int Forum Allergy Rhinol*. 2016;6(5):518-522.

31. Anzarut A, Olson J, Singh P, Rowe BH, Tredget EE. The effectiveness of pressure garment therapy for the prevention of abnormal scarring after burn injury: a meta-analysis. *Journal of Plastic, Reconstructive & Aesthetic Surgery*. 2009;62(1):77-84.
32. Golladay ES. Treatment of keloids by single intraoperative perilesional injection of repository steroid. *Southern medical journal*. 1988;81(6):736-738.
33. España A, Solano T, Quintanilla E. Bleomycin in the treatment of keloids and hypertrophic scars by multiple needle punctures. *Dermatologic Surgery*. 2001;27(1):23-27.
34. Goldman MP, Fitzpatrick RE. Laser Treatment of Scars. *Dermatologic Surgery*. 1995;21(8):685-687.
35. Lee JP, Jalili RB, Tredget EE, Demare JR, Ghahary A. Short Communication: Antifibrogenic Effects of Liposome-Encapsulated IFN- $\alpha$ 2b Cream on Skin Wounds in a Fibrotic Rabbit Ear Model. *Journal of Interferon & Cytokine Research*. 2005;25(10):627-631.
36. O'Brien L, Jones DJ. Silicone gel sheeting for preventing and treating hypertrophic and keloid scars. *Cochrane Database of Systematic Reviews*. 2013(9).
37. Barril S, Alonso A, Rodriguez-Portal JA, et al. Palliative Care in Diffuse Interstitial Lung Disease: Results of a Spanish Survey. *Arch Bronconeumol*. 2018;54(3):123-127.
38. Karagoz H, Yuksel F, Ulkur E, Evinc R. Comparison of efficacy of silicone gel, silicone gel sheeting, and topical onion extract including heparin and allantoin for the treatment of postburn hypertrophic scars. *Burns*. 2009;35(8):1097-1103.
39. Atiyeh BS. Nonsurgical management of hypertrophic scars: evidence-based therapies, standard practices, and emerging methods. *Aesthetic Plast Surg*. 2007;31(5):468-492; discussion 493-464.
40. Manuskiatti W, Fitzpatrick RE. Treatment response of keloidal and hypertrophic sternotomy scars: comparison among intralesional corticosteroid, 5-fluorouracil, and 585-nm flashlamp-pumped pulsed-dye laser treatments. *Archives of dermatology*. 2002;138(9):1149-1155.
41. George W. Linear lymphatic hypopigmentation after intralesional corticosteroid injection: report of two cases. *Cutis*. 1999;64(1):61-64.
42. Cabral JD, McConnell MA, Fitzpatrick C, et al. Characterization of the in vivo host response to a bi-labeled chitosan-dextran based hydrogel for postsurgical adhesion prevention. *Journal of biomedical materials research Part A*. 2015;103(8):2611-2620.
43. Rajiv S, Harding M, Bassiouni A, et al. The efficacy and safety of chitosan dextran gel in a burr hole neurosurgical sheep model. *Acta Neurochir (Wien)*. 2013;155(7):1361-1366; discussion 1366.

44. Valentine R, Athanasiadis T, Moratti S, Hanton L, Robinson S, Wormald PJ. The efficacy of a novel chitosan gel on hemostasis and wound healing after endoscopic sinus surgery. *Am J Rhinol Allergy*. 2010;24(1):70-75.
45. Ha T, Valentine R, Moratti S, Hanton L, Robinson S, Wormald PJ. The efficacy of a novel budesonide chitosan gel on wound healing following endoscopic sinus surgery. *Int Forum Allergy Rhinol*. 2017.
46. Ngoc Ha T, Valentine R, Moratti S, Robinson S, Hanton L, Wormald PJ. A blinded randomized controlled trial evaluating the efficacy of chitosan gel on ostial stenosis following endoscopic sinus surgery. *Int Forum Allergy Rhinol*. 2013;3(7):573-580.
47. Wedmore I, McManus JG, Pusateri AE, Holcomb JB. A special report on the chitosan-based hemostatic dressing: experience in current combat operations. *Journal of Trauma and Acute Care Surgery*. 2006;60(3):655-658.
48. <https://www.tricolbiomedical.com/about/>.
49. Valentine R, Athanasiadis T, Moratti S, Robinson S, Wormald PJ. The efficacy of a novel chitosan gel on hemostasis after endoscopic sinus surgery in a sheep model of chronic rhinosinusitis. *Am J Rhinol Allergy*. 2009;23(1):71-75.
50. Valentine R, Wormald PJ. Nasal dressings after endoscopic sinus surgery: what and why? *Curr Opin Otolaryngol Head Neck Surg*. 2010;18(1):44-48.
51. Valentine R, Boase S, Jervis-Bardy J, Dones Cabral JD, Robinson S, Wormald PJ. The efficacy of hemostatic techniques in the sheep model of carotid artery injury. *Int Forum Allergy Rhinol*. 2011;1(2):118-122.
52. <https://www.merit.com/cardiac-intervention/hemostasis/compression-devices/clo-sur-p-a-d-clo-sur-plus-p-a-d/>.
53. Kjaer P, Kongsted A, Ris I, et al. GLA:D((R)) Back group-based patient education integrated with exercises to support self-management of back pain - development, theories and scientific evidence. *BMC Musculoskelet Disord*. 2018;19(1):418.
54. <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm?ID=K021062>.
55. <https://syvek.com/syveknt/>.
56. Jewelewicz DD, Cohn SM, Crookes BA, Proctor KG. Modified Rapid Deployment Hemostat Bandage Reduces Blood Loss and Mortality in Coagulopathic Pigs with Severe Liver Injury. *Journal of Trauma and Acute Care Surgery*. 2003;55(2):275-281.
57. Gegel BT, Burgert JM, Lockhart C, et al. Effects of Celox and TraumaDEX on hemorrhage control in a porcine model. *AANA J*. 2010;78(2):115-120.

58. Akiyama K, Karaki M, Yonezaki M, et al. Usefulness of nasal packing with silver-containing carboxy methylated cellulose in endonasal sinus surgery. *Auris Nasus Larynx*. 2014;41(3):264-268.
59. Diamond MP, Luciano A, Johns DA, Dunn R, Young P, Bieber E. Reduction of postoperative adhesions by N, O-carboxymethylchitosan: a pilot study. *Fertility and sterility*. 2003;80(3):631-636.
60. Kennedy R, Costain DJ, McAlister VC, Lee TD. Prevention of experimental postoperative peritoneal adhesions by N, O-carboxymethyl chitosan. *Surgery*. 1996;120(5):866-870.
61. Costain DJ, Kennedy R, Ciona C, McAlister VC, Lee TDG. Prevention of postsurgical adhesions with N,O-carboxymethyl chitosan: Examination of the most efficacious preparation and the effect of N,O-carboxymethyl chitosan on postsurgical healing. *Surgery*. 1997;121(3):314-319.
62. Vlahos A, Yu P, Lucas CE, Ledgerwood AM. Effect of a composite membrane of chitosan and poloxamer gel on postoperative adhesive interactions. *The American surgeon*. 2001;67(1):15-21.
63. Zhang Z-L, Xu S-W, Zhou X-L. Preventive effects of chitosan on peritoneal adhesion in rats. *World Journal of Gastroenterology: WJG*. 2006;12(28):4572.
64. Kaats GR, Michalek JE, Preuss HG. Evaluating efficacy of a chitosan product using a double-blinded, placebo-controlled protocol. *Journal of the American College of Nutrition*. 2006;25(5):389-394.
65. Wuolioki E, Hirvela T, Ylitalo P. Decrease in serum LDL cholesterol with microcrystalline chitosan. *Methods and findings in experimental and clinical pharmacology*. 1999;21(5):357-362.
66. Bokura H, Kobayashi S. Chitosan decreases total cholesterol in women: a randomized, double-blind, placebo-controlled trial. *European journal of clinical nutrition*. 2003;57(5):721-725.
67. Landes D, Bough W. Effects of chitosan—a coagulating agent for food processing wastes—in the diets of rats on growth and liver and blood composition. *Bulletin of environmental contamination and toxicology*. 1976;15(5):555-563.
68. Vahouny GV, Satchithanandam S, Cassidy MM, Lightfoot FB, Furda I. Comparative effects of chitosan and cholestyramine on lymphatic absorption of lipids in the rat. *The American journal of clinical nutrition*. 1983;38(2):278-284.
69. Fukada Y, Kimura K, Ayaki Y. Effect of chitosan feeding on intestinal bile acid metabolism in rats. *Lipids*. 1991;26(5):395-399.
70. LeHoux J, Grondin F. Some effects of chitosan on liver function in the rat. *Endocrinology*. 1993;132(3):1078-1084.

71. Deuchi K, Kanauchi O, Shizukuishi M, Kobayashi E. Continuous and massive intake of chitosan affects mineral and fat-soluble vitamin status in rats fed on a high-fat diet. *Bioscience, biotechnology, and biochemistry*. 1995;59(7):1211-1216.
72. Razdan A, Pettersson D, Pettersson J. Broiler chicken body weights, feed intakes, plasma lipid and small-intestinal bile acid concentrations in response to feeding of chitosan and pectin. *British Journal of Nutrition*. 1997;78(2):283-291.
73. Ormrod DJ, Holmes CC, Miller TE. Dietary chitosan inhibits hypercholesterolaemia and atherogenesis in the apolipoprotein E-deficient mouse model of atherosclerosis. *Atherosclerosis*. 1998;138(2):329-334.
74. Illum L, Jabbal-Gill I, Hinchcliffe M, Fisher A, Davis S. Chitosan as a novel nasal delivery system for vaccines. *Advanced drug delivery reviews*. 2001;51(1-3):81-96.
75. Rasmussen RS, Morrissey MT. Chitin and chitosan. *Marine nutraceuticals and functional foods*. 2007;7:155-182.
76. Mhurchu CN, Dunshea - Mooij C, Bennett D, Rodgers A. Effect of chitosan on weight loss in overweight and obese individuals: a systematic review of randomized controlled trials. *Obesity reviews*. 2005;6(1):35-42.
77. Athanasiadis T, Beule AG, Robinson BH, Robinson SR, Shi Z, Wormald PJ. Effects of a novel chitosan gel on mucosal wound healing following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis. *Laryngoscope*. 2008;118(6):1088-1094.
78. Ha T, Valentine R, Moratti S, Hanton L, Robinson S, Wormald PJ. The efficacy of a novel budesonide chitosan gel on wound healing following endoscopic sinus surgery. *Int Forum Allergy Rhinol*. 2018;8(3):435-443.
79. Bae K, Jun E, Lee S, Paik D, Kim J. Effect of water-soluble reduced chitosan on *Streptococcus mutans*, plaque regrowth and biofilm vitality. *Clinical oral investigations*. 2006;10(2):102.
80. Paramasivan S, Jones D, Baker L, et al. The use of chitosan-dextran gel shows anti-inflammatory, antibiofilm, and antiproliferative properties in fibroblast cell culture. *Am J Rhinol Allergy*. 2014;28(5):361-365.
81. Olivieri NF, Brittenham GM, McLaren CE, et al. Long-term safety and effectiveness of iron-chelation therapy with deferiprone for thalassemia major. *New England Journal of Medicine*. 1998;339(7):417-423.
82. Food U, Administration D. FDA approves Ferriprox to treat patients with excess iron in the body. In:2011.
83. ten Raa S, Van den Tol MP, Sluiter W, Hofland LJ, van Eijck CH, Jeekel H. The role of neutrophils and oxygen free radicals in post-operative adhesions. *Journal of Surgical Research*. 2006;136(1):45-52.

84. Wallace DF. The Regulation of Iron Absorption and Homeostasis. *Clin Biochem Rev.* 2016;37(2):51-62.
85. Ramezanpour M, Smith JLP, Ooi ML, et al. Deferiprone has anti-inflammatory properties and reduces fibroblast migration in vitro. *Scientific Reports.* 2019;9(1):2378.
86. Barton JC. Drug evaluation: deferitritin for iron overload disorders. *IDrugs.* 2007;10(7):480-490.
87. Benoit-Biancamano M-O, Connelly J, Villeneuve L, Caron P, Guillemette C. Deferiprone glucuronidation by human tissues and recombinant UDP glucuronosyltransferase 1A6: an in vitro investigation of genetic and splice variants. *Drug Metabolism and Disposition.* 2009;37(2):322-329.
88. Wood JC, Otto-Duessel M, Gonzalez I, et al. Deferasirox and deferiprone remove cardiac iron in the iron-overloaded gerbil. *Translational research.* 2006;148(5):272-280.
89. Lund JM, Broliden K, Pyra MN, et al. Correction for Lund et al., HIV-1-Neutralizing IgA Detected in Genital Secretions of Highly HIV-1-Exposed Seronegative Women on Oral Preexposure Prophylaxis. *J Virol.* 2017;91(3).
90. Stojiljkovic I, Kumar V, Srinivasan N. Non-iron metalloporphyrins: potent antibacterial compounds that exploit haem/Hb uptake systems of pathogenic bacteria. *Mol Microbiol.* 1999;31(2):429-442.
91. Le T, Psaltis A, Tan LW, Wormald PJ. The efficacy of topical antibiofilm agents in a sheep model of rhinosinusitis. *Am J Rhinol.* 2008;22(6):560-567.
92. Goss CH, Kaneko Y, Khoo L, et al. Gallium disrupts bacterial iron metabolism and has therapeutic effects in mice and humans with lung infections. *Science translational medicine.* 2018;10(460).
93. Frezza M, Schmitt S, Tomco D, Chen D, Verani CN, Dou QP. Gallium(III) Complexes, Inhibition of Proteasome Activity. In: Kretsinger RH, Uversky VN, Permyakov EA, eds. *Encyclopedia of Metalloproteins.* New York, NY: Springer New York; 2013:819-822.
94. <http://aridispharmaceuticals.com/panaecin.html>.
95. Chitambar CR. The therapeutic potential of iron-targeting gallium compounds in human disease: From basic research to clinical application. *Pharmacological research.* 2017;115:56-64.
96. Richter K, Ramezanpour M, Thomas N, Prestidge CA, Wormald PJ, Vreugde S. Mind "De GaPP": in vitro efficacy of deferiprone and gallium-protoporphyrin against *Staphylococcus aureus* biofilms. *Int Forum Allergy Rhinol.* 2016;6(7):737-743.



97. Ooi ML, Richter K, Drilling AJ, et al. Safety and Efficacy of Topical Chitogel-Deferiprone-Gallium Protoporphyrin in Sheep Model. *Front Microbiol.* 2018;9:917.
98. Fokkens WJ, Lund VJ, Mullol J, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2012. *Rhinol Suppl.* 2012;23:3 p preceding table of contents, 1-298.
99. Benninger MS, Ferguson BJ, Hadley JA, et al. Adult chronic rhinosinusitis: definitions, diagnosis, epidemiology, and pathophysiology. *Otolaryngology-Head and Neck Surgery.* 2003;129(3):S1-S32.
100. Meltzer EO HD, Hadley JA, Lanza DC, Marple BF, Nicklas RA Rhinosinusitis: establishing definitions for clinical research and patient care. *J Allergy Clin Immunol* 2004;114:155-212.
101. Fokkens WJ, Lund VJ, Hopkins C, et al. European position paper on rhinosinusitis and nasal polyps 2020. *Rhinology.* 2020;58:I.
102. Cooke G, Valenti L, Glasziou P, Britt H. Common general practice presentations and publication frequency. *Australian Family Physician.* 2013;42:65-68.
103. Hastan D, Fokkens WJ, Bachert C, et al. Chronic rhinosinusitis in Europe – an underestimated disease. A GA2LEN study. *Allergy.* 2011;66(9):1216-1223.
104. JG C. Prevalence of selected chronic conditions. *Vital Health Stat 10.* 1990-1992;1997 Jan;(194):1-89.
105. Y.S. Kim NHK, S.Y. Seong, K.R. Kim, G.B. Lee, K.S. Kim. Prevalence and risk factors of chronic rhinosinusitis in Korea. *Am J Rhinol Allergy.* 2011;25 , pp. 117-121.
106. ABS. Australian Health, Survey, <https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0012014-15?OpenDocument>.
107. Bhattacharyya N. Incremental health care utilization and expenditures for chronic rhinosinusitis in the United States. *Ann Otol Rhinol Laryngol.* 2011;120:423-427.
108. Aramani A, Karadi R, Kumar S. A study of anatomical variations of osteomeatal complex in chronic rhinosinusitis patients-CT findings. *Journal of clinical and diagnostic research: JCDR.* 2014;8(10):KC01.
109. Wang X, Moylan B, Leopold DA, Kim J et al. Mutation in the gene responsible for cystic fibrosis and predisposition to chronic rhinosinusitis in the general population. *JAMA.* 2000 284(14):1814-9(0098-7484 (Print)).
110. Zariwala MA, Knowles MR, Omran H. Genetic Defects in Ciliary Structure and Function. *Annual Review of Physiology.* 2007;69(1):423-450.
111. Slavin RG, Spector SL, Bernstein IL, et al. The diagnosis and management of sinusitis: A practice parameter update. *Journal of Allergy and Clinical Immunology.* 2005;116(6):S13-S47.

112. Kern RC, Conley DB, Walsh W, et al. Perspectives on the etiology of chronic rhinosinusitis: an immune barrier hypothesis. *Am J Rhinol.* 2008;22(6):549-559.
113. Soyka MB, Wawrzyniak P, Eiwegger T, et al. Defective epithelial barrier in chronic rhinosinusitis: the regulation of tight junctions by IFN-gamma and IL-4. *J Allergy Clin Immunol.* 2012;130(5):1087-1096 e1010.
114. Cantin AM, Hanrahan JW, Bilodeau G, et al. Cystic fibrosis transmembrane conductance regulator function is suppressed in cigarette smokers. *Am J Respir Crit Care Med.* 2006;173(10):1139-1144.
115. Wolf C. Urban air pollution and health: an ecological study of chronic rhinosinusitis in Cologne, Germany. *Health Place.* 2002;8(2):129-139.
116. Kim J, Hanley JA. The role of woodstoves in the etiology of nasal polyposis. *Arch Otolaryngol Head Neck Surg.* 2002;128(6):682-686.
117. Psaltis AJ, Ha KR, Beule AG, Tan LW, Wormald PJ. Confocal scanning laser microscopy evidence of biofilms in patients with chronic rhinosinusitis. *Laryngoscope.* 2007;117(7):1302-1306.
118. Psaltis AJ, Weitzel EK, Ha KR, Wormald PJ. The effect of bacterial biofilms on post-sinus surgical outcomes. *Am J Rhinol.* 2008;22(1):1-6.
119. Stephenson MF, Mfuna L, Dowd SE, et al. Molecular characterization of the polymicrobial flora in chronic rhinosinusitis. *Journal of otolaryngology - head & neck surgery = Le Journal d'oto-rhino-laryngologie et de chirurgie cervico-faciale.* 2010;39(2):182-187.
120. Wald ER. The microbiology of chronic sinusitis in children. A review. *Acta Otorhinolaryngol Belg.* 1997;51(1):51-54.
121. Boase S, Foreman A, Cleland E, et al. The microbiome of chronic rhinosinusitis: culture, molecular diagnostics and biofilm detection. *BMC Infect Dis.* 2013;13:210.
122. Bachert C, Gevaert P, Holtappels G, Johansson Sg, van Cauwenberge P. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol.* 2001 107(4):607-14(0091-6749 (Print)).
123. Ramakrishnan Y, Shields RC, Elbadawey MR, Wilson JA. Biofilms in chronic rhinosinusitis: what is new and where next? *J Laryngol Otol.* 2015;129(8):744-751.
124. Cryer J, Schipor I, Perloff Jr, Palmer JN,. Evidence of bacterial biofilms in human chronic sinusitis. *ORL J Otorhinolaryngol Relat Spec.* 2004;66(3):155-8(0301-1569 (Print)).
125. Foreman A, Jervis-Bardy J, Wormald PJ. Do biofilms contribute to the initiation and recalcitrance of chronic rhinosinusitis? *Laryngoscope.* 2011;121(5):1085-1091.

126. Psaltis AJ, Weitzel EK, Ha KR, Wormald P-J. The Effect of Bacterial Biofilms on Post-sinus Surgical Outcomes. *American Journal of Rhinology*. 2008;22(1):1-6.
127. Li H, Wang D, Sun X, Hu L, Yu H, Wang J. Relationship between bacterial biofilm and clinical features of patients with chronic rhinosinusitis. *European Archives of Oto-Rhino-Laryngology*. 2012;269(1):155-163.
128. Braun H, Buzina W, Freudenschuss K, Beham, Stammberger H. 'Eosinophilic fungal rhinosinusitis': a common disorder in Europe? *Laryngoscope*. 2003 113(2):264-9(0023-852X (Print)).
129. Weschta M, Rimek D, Formanek M, Polzehl D, Podbielski A, Riechelmann H. Topical antifungal treatment of chronic rhinosinusitis with nasal polyps: a randomized, double-blind clinical trial. *J Allergy Clin Immunol*. 2004;113(6):1122-1128.
130. Singh NK, Garg LN, Shekhar S, et al. Fungi in association with chronic bacterial rhinosinusitis; relationship with a synergistic effect: a prospective study. *International Journal of Otorhinolaryngology and Head and Neck Surgery; Vol 4, No 5 (2018): September-October 2018*. 2018.
131. Goggin RK, Bennett CA, Bialasiewicz S, et al. The presence of virus significantly associates with chronic rhinosinusitis disease severity. *Allergy*. 2019.
132. Ramadan HH, Farr RW, Wetmore SJ. Adenovirus and respiratory syncytial virus in chronic sinusitis using polymerase chain reaction. *Laryngoscope*. 1997;107(7):923-925.
133. Naidoo Y, Tan N, Singhal D, Wormald PJ. Chronic rhinosinusitis assessment using the Adelaide Disease Severity Score. *J Laryngol Otol*. 2013 127 Suppl 2:S24-8(1748-5460 (Electronic)).
134. Doulaptsi M, Prokopakis E, Seys S, Pugin B, Steelant B, Hellings P. Visual analogue scale for sino-nasal symptoms severity correlates with sino-nasal outcome test 22: paving the way for a simple outcome tool of CRS burden. *Clin Transl Allergy*. 2018;8:32.
135. Hopkins C, Browne Jp, Slack R, Lund V, Brown P. The Lund-Mackay staging system for chronic rhinosinusitis: how is it used and what does it predict? *Otolaryngol Head Neck Surg*. 2007;137(4):555-61(0194-5998 (Print)).
136. Psaltis AJ, Li G, Vaezeafshar R, Cho K-S, Hwang PH. Modification of the lund-kennedy endoscopic scoring system improves its reliability and correlation with patient-reported outcome measures. *The Laryngoscope*. 2014;124(10):2216-2223.
137. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science (New York, NY)*. 1999;284(5418):1318-1322.
138. Orlandi RR KT, Hwang PH, et al. International consensus statement on allergy and rhinology: rhinosinusitis. *Int Forum Allergy Rhinol*. 2016; 6(Suppl 1):S22–209.

139. Harvey R HS, Badia L, et al. Nasal saline irrigations for the symptoms of chronic rhinosinusitis. *Cochrane Database Syst Rev*. 2007 Jul 18;(3):CD006394.
140. Chong LY HK, Hopkins C, et al. Different types of intranasal steroids for chronic rhinosinusitis. *Cochrane Database Syst Rev*. 2016;4:CD011993.
141. Kanjanawasee D SK, Chitsuthipakorn W, et al. Hypertonic saline versus isotonic saline nasal irrigation: systematic review and meta-analysis. *Am J Rhinol Allergy*. 2018;32:269–279.
142. Sastre J MR. Local and systemic safety of intranasal corticosteroids. *J Investig Allergol Clin Immunol*. 2012;22:1–12.
143. Tait S KD, Suko J, et al. Effect of budesonide added to large-volume, low-pressure saline sinus irrigation for chronic rhinosinusitis: A randomized clinical trial. *JAMA Otolaryngol Head Neck Surg*. 2018;144:605–612.
144. Soudry E WJ, Vaezeafshar R, et al. Safety analysis of long-term budesonide nasal irrigations in patients with chronic rhinosinusitis post endoscopic sinus surgery. *Int Forum Allergy Rhinol*. 2016;6:568–572.
145. Smith KA FG, Mechor B, et al. Safety of long-term highvolume sinonasal budesonide irrigations for chronic rhinosinusitis. *Int Forum Allergy Rhinol*. 2016;6:228–232.
146. ThomasWWIII HR, Rudmik L, Hwang PH, Schlosser RJ. Distribution of topical agents to the paranasal sinuses: an evidence-based review with recommendations. *Int Forum Allergy Rhinol*. 2013;3(9):691-703.
147. Rudmik L HM, Schlosser RJ, et al. Topical therapies in the management of chronic rhinosinusitis: an evidence-based review with recommendations. *Int Forum Allergy Rhinol* 2013;3 (4):281-298.
148. Soler ZM OS, Kern RC, et al. Antimicrobials and chronic rhinosinusitis with or without polyposis in adults: an evidenced-based review with recommendations. *Int Forum Allergy Rhinol*. 2013;3 (1):31-47.
149. Lim M C, Leong JL. T. Topical antimicrobials in the management of chronic rhinosinusitis: a systematic review. *Am J Rhinol*. 2008;22(4):381-389.
150. Wei CC AN, Cohen NA. Use of topical nasal therapies in the management of chronic rhinosinusitis. *Laryngoscope*. 2013;123(10):2347-2359.
151. Jervis-Bardy J BS, Psaltis A, Foreman A, Wormald PJ. A randomized trial of mupirocin sinonasal rinses versus saline in surgically recalcitrant staphylococcal chronic rhinosinusitis. *Laryngoscope*. 2012;122(10):2148-2153.
152. Rudmik L, Hoy M, Schlosser RJ, Harvey RJ, et al. Topical therapies in the management of chronic rhinosinusitis: an evidence-based review with

- recommendations. *Int Forum Allergy Rhinol.* 2013 3(4):281-98(2042-6984 (Electronic)).
153. HeadK C, PiromchaiP, et al. Systemic and topical antibiotics for chronic rhinosinusitis. *Cochrane Database Syst Rev.* 2016;4:CD011994.
154. Bulbul T BO, Guclu O, et al. Effect of glucocorticoids on nasal polyposis, with detection of inflammatory response by measurement of nitric oxide levels in nasal polyp tissue. *J Laryngol Otol.* 2013;127:584–589.
155. Leung RM DK, Smith TL. When do the risks of repeated courses of corticosteroids exceed the risks of surgery? *Int Forum Allergy Rhinol.* 2014;4:871–876.
156. Smith SS EC, Tan BK, et al. National burden of antibiotic use for adult rhinosinusitis. *J Allergy Clin Immunol.* 2013;132:1230–1232.
157. HeadK C, PiromchaiP. Systemic and topical antibiotics for chronic rhinosinusitis. *Cochrane Database Syst Rev.* 2016;4:CD011994.
158. Seresirikachorn K SN, Srisunthornphanich C, et al. Factors of success of low-dose macrolides in chronic sinusitis: systematic review and meta-analysis. *Laryngoscope.* 2019;129:1510–1519.
159. Harvey RJ WB, Lund VJ. Anti-inflammatory effects of macrolides: applications in chronic rhinosinusitis. *Immunol Allergy Clin North Am.* 2009;29:689–703.
160. Mosholder AD MJ, Alexander JJ, et al. Cardiovascular risks with azithromycin and other antibacterial drugs. *N Engl J Med.* 2013;368:1665–1668.
161. Head K SS, Chong LY, et al. Topical and systemic antifungal therapy for chronic rhinosinusitis. *Cochrane Database Syst Rev.* 2018;9:CD012453.
162. Rosenfeld RM, Piccirillo JF, Chandrasekhar SS, et al. Clinical Practice Guideline (Update): Adult Sinusitis. *Otolaryngology–Head and Neck Surgery.* 2015;152(2\_suppl):S1-S39.
163. Stephen S, Subashini B, Thomas R, Philip A, Sundaresan R. Skull Base Osteomyelitis Caused by an Elegant Fungus. *The Journal of the Association of Physicians of India.* 2016;64(2):70-71.
164. Sacks PL, Harvey R, Rimmer J, Gallagher R, Sacks R. Topical and systemic antifungal therapy for the symptomatic treatment of chronic rhinosinusitis. *Cochrane Database Syst Rev.* 2011 10;(8):CD008263(1469-493X (Electronic)).
165. Tsetsos N, Goudakos JK, Daskalakis D, Konstantinidis I, Markou K. Monoclonal antibodies for the treatment of chronic rhinosinusitis with nasal polyposis: a systematic review. *Rhinology.* 2018;56(1):11-21.
166. Pinto JM MN, DiTineo M, et al. A randomized, double-blind, placebo-controlled trial of anti-ige for chronic rhinosinusitis. *Rhinology.* 2010;48:318–324.

167. Gevaert P CL, Van Zele T, et al. Omalizumab is effective in allergic and nonallergic patients with nasal polyps and asthma. *J Allergy Clin Immunol*. 2013;131:110–116 e111.
168. Gevaert P L-LD, Lackner A, et al. Nasal il-5 levels determine the response to anti-il-5 treatment in patients with nasal polyps. *J Allergy Clin Immunol*. 2006;118:1133–1141.
169. Bachert C SA, Lund VJ, et al. Reduced need for surgery in severe nasal polyposis with mepolizumab: randomized trial. *J Allergy Clin Immunol*. 2017;140:1024–1031 e1014.
170. Bachert C HJ, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled,, (10209):1638-1650 p-gptLN.
171. Hellings PW FW, Bachert C, et al. Positioning the principles of precision medicine in care pathways for allergic rhinitis and chronic rhinosinusitis - A EUFOREA-ARIA-EPOS AIRWAYS ICP statement. *Allergy*. 2017;72:1297–1305.
172. Scangas GA, Wu AW, Ting JY, et al. Cost utility analysis of dupilumab versus endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps. *The Laryngoscope*. 2020.
173. Bhattacharyya N. The economic burden and symptom manifestations of chronic rhinosinusitis. *Am J Rhinol*. 2003 17(1):27-32(1050-6586 (Print)).
174. Cain RB, Lal D. Update on the management of chronic rhinosinusitis. *Infect Drug Resist*. 2013;6:1-14(1178-6973 (Print)).
175. Gonzales R, Bartlett JG, Besser RE, Hickner JM, Hoffman JR, Sande MA. Principles of appropriate antibiotic use for treatment of nonspecific upper respiratory tract infections in adults: Background. *Annals of Emergency Medicine*. 2001;37(6):698-702.
176. Zarogoulidis P, Papanas N, Kioumis I, Chatzaki E, Maltezos E, Zarogoulidis K. Macrolides: from in vitro anti-inflammatory and immunomodulatory properties to clinical practice in respiratory diseases. *Eur J Clin Pharmacol*. 2012 68(5):479-503(1432-1041 (Electronic)).
177. Orlandi RR, Kingdom TT, Hwang PH, et al. International Consensus Statement on Allergy and Rhinology: Rhinosinusitis. *Int Forum Allergy Rhinol*. 2016 Suppl 1:S22-209.(2042-6984 (Electronic)).
178. Landolina N, Levi-Schaffer F. Monoclonal antibodies: the new magic bullets for allergy: IUPHAR Review 17. *Br J Pharmacol*. 2016 173(5):793-803(1476-5381 (Electronic)).

179. Drilling AJ, Ooi ML, Miljkovic D, et al. Long-Term Safety of Topical Bacteriophage Application to the Frontal Sinus Region. *Frontiers in cellular and infection microbiology*. 2017;7:49-49.
180. Fong SA, Drilling A, Morales S, et al. Activity of Bacteriophages in Removing Biofilms of *Pseudomonas aeruginosa* Isolates from Chronic Rhinosinusitis Patients. *Frontiers in cellular and infection microbiology*. 2017;7:418-418.
181. Ooi ML, Jane Drilling A, Morales S, et al. Safety and Tolerability of Bacteriophage Therapy for Chronic Rhinosinusitis Due to *Staphylococcus aureus*. *JAMA Otolaryngol Head Neck Surg*. 2019 Jun 20.
182. Cleland EJ, Drilling A, Bassiouni A, James C, Vreugde S, Wormald PJ. Probiotic manipulation of the chronic rhinosinusitis microbiome. *Int Forum Allergy Rhinol*. 2014;4(4):309-314.
183. Fairley MJW, Fairley MSA. Patrick Watson-Williams and the concept of focal sepsis in the sinuses. *Medical Information*. 1233:642244.
184. Roh SW, Kim DH, Cardoso AC, Fessler RG. Endoscopic Foraminotomy Using MED System in Cadaveric Specimens. *Spine*. 2000;25(2):260.
185. Lund VJ, Stammberger H, Nicolai P, et al. European position paper on endoscopic management of tumours of the nose, paranasal sinuses and skull base. *Rhinol Suppl*. 2010;22:1-143.
186. Wormald PJ. Surgery of the frontal recess and frontal sinus. *Rhinology*. 2005;43(2):82-85.
187. Lund VJ. Outcomes in rhinology. *Rhinology*. 2009;47(1):1-2.
188. Kennedy DW, Senior BA. Endoscopic sinus surgery. A review. *Otolaryngologic Clinics of North America*. 1997;30(3):313-330.
189. Chen PG, Wormald PJ, Payne SC, Gross WE, Gross CW. A golden experience: Fifty years of experience managing the frontal sinus. *Laryngoscope*. 2016;126(4):802-807.
190. Metson RB, Gliklich RE. Clinical outcomes in patients with chronic sinusitis. *The Laryngoscope*. 2000;110(3 Pt 3):24.
191. Smith TL. Outcomes of endoscopic sinus surgery for chronic rhinosinusitis associated with sinonasal polyposis. *American Journal of Rhinology*. 2007;21(1):84-88.
192. Hannley M. Evidence Supporting Endoscopic Sinus Surgery in the Management of Adult Chronic Rhinosinusitis: A Systematic Review. *American Journal of Rhinology*. 2005;19(6):537-543.
193. Vashishta R, Soler ZM, Nguyen SA, Schlosser RJ. A systematic review and meta - analysis of asthma outcomes following endoscopic sinus surgery for chronic

- rhinosinusitis. In. *International forum of allergy & rhinology*. Vol 3: Wiley Online Library; 2013:788-794.
194. Samargandy S, Grose E, Chan Y, Monteiro E, Lee JM, Yip J. Medical and surgical treatment outcomes in patients with chronic rhinosinusitis and immunodeficiency: a systematic review. In. *International Forum of Allergy & Rhinology*: Wiley Online Library; 2020.
195. Loftus CA, Soler ZM, Koochakzadeh S, et al. Revision surgery rates in chronic rhinosinusitis with nasal polyps: meta - analysis of risk factors. In. *International Forum of Allergy & Rhinology*. Vol 10: Wiley Online Library; 2020:199-207.
196. Chen S, Zhou A, Emmanuel B, Garcia D, Rosta E. Systematic literature review of humanistic and economic burdens of chronic rhinosinusitis with nasal polyposis. *Current Medical Research and Opinion*. 2020(just-accepted):1-1.
197. Alanin MC, Hopkins C. Effect of Functional Endoscopic Sinus Surgery on Outcomes in Chronic Rhinosinusitis. *Current Allergy and Asthma Reports*. 2020;20(7):27-27.
198. Krings JG, Kallogjeri D, Wineland A, Nepple KG, Piccirillo JF, Getz AE. Complications of primary and revision functional endoscopic sinus surgery for chronic rhinosinusitis. *The Laryngoscope*. 2014;124(4):838-845.
199. Durmaz A, Gerek M. Complications of Endoscopic Sinus Surgery. In: Cingi C, Bayar Muluk N, eds. *All Around the Nose: Basic Science, Diseases and Surgical Management*. Cham: Springer International Publishing; 2020:579-585.
200. Messerklinger W. On the drainage of the normal frontal sinus of man. *Acta otolaryngologica*. 1967;63(2-3):176-181.
201. Knowlton CD, McGregor G. How and when the mucous membrane of the maxillary sinus regenerates: an experimental study in the dog. *Archives of Otolaryngology*. 1928;8(6):647-656.
202. Marks SC. Acute sinusitis in the rabbit: a new rhinogenic model. *The Laryngoscope*. 1997;107(12):1579-1585.
203. Schenck NL. Frontal sinus disease. I. An historical perspective on research. *The Laryngoscope*. 1974;84(6):1031-1044.
204. Hilding A. Experimental surgery of the nose and sinuses: II. Gross results following the removal of the intersinus septum and of strips of mucous membrane from the frontal sinus of the dog. *Archives of Otolaryngology*. 1933;17(3):321-327.
205. Hilding A, Banovetz J. Occluding scars in the sinuses: relation to bone growth. *The Laryngoscope*. 1963;73(9):1201-1218.
206. Kanda T, Hilding D. Development of respiratory tract cilia in fetal rabbits: electron microscopic investigation. *Acta oto-laryngologica*. 1968;65(6):611-624.



207. Weber R, Keerl R, Jaspersen D, Huppmann A, Schick B, Draf W. Computer-assisted documentation and analysis of wound healing of the nasal and oesophageal mucosa. *The Journal of Laryngology & Otology*. 1996;110(11):1017-1021.
208. Weber R, Keerl R. Healing in the nasal mucosa. *Journal of wound care*. 1998;7(2):101-102.
209. Bradley DT, Kountakis SE. Role of Interleukins and Transforming Growth Factor -  $\beta$  in Chronic Rhinosinusitis and Nasal Polyposis. *The Laryngoscope*. 2005;115(4):684-686.
210. Thaler ER. Postoperative care after endoscopic sinus surgery. *Archives of Otolaryngology-Head & Neck Surgery*. 2002;128(10):1204-1206.
211. Stamberger H. Endoscopic endonasal surgery-Concepts in treatment of recurring rhinosinusitis. *Otolaryngol Head & Neck Surgery*. 1986;94:143-156.
212. Levine HL. Functional endoscopic sinus surgery: evaluation, surgery, and follow - up of 250 patients. *The Laryngoscope*. 1990;100(1):79-84.
213. Shrime MG, Tabae A, Hsu AK, Rickert S, Close LG. Synechia formation after endoscopic sinus surgery and middle turbinate medialization with and without FloSeal. *American journal of rhinology*. 2007;21(2):174-179.
214. Ramadan HH. Surgical causes of failure in endoscopic sinus surgery. *The Laryngoscope*. 1999;109(1):27-29.
215. Jacobs JB. 100 years of frontal sinus surgery. *The Laryngoscope*. 1997;107(S83):1-36.
216. Demo MM, Steed MB. Chapter 35 - Traumatic Epistaxis. In: Bagheri SC, Bell RB, Khan HA, eds. *Current Therapy In Oral and Maxillofacial Surgery*. Saint Louis: W.B. Saunders; 2012:281-288.
217. Merrick MA. 8 - Therapeutic Modalities As an Adjunct to Rehabilitation. In: Andrews JR, Harrelson GL, Wilk KE, eds. *Physical Rehabilitation of the Injured Athlete (Fourth Edition)*. Philadelphia: W.B. Saunders; 2012:104-142.
218. Valentine R, Wormald PJ, Sindwani R. Advances in absorbable biomaterials and nasal packing. *Otolaryngol Clin North Am*. 2009;42(5):813-828, ix.
219. Weber RK. Nasal packing and stenting. *GMS current topics in otorhinolaryngology, head and neck surgery*. 2009;8.
220. Weber R, Hay U. Is packing of the nose up-to-date? *Laryngo-rhino-otologie*. 2003;82(9):650-654.
221. Tzelnick S, Alkan U, Leshno M, Hwang P, Soudry E. Sinonasal debridement versus no debridement for the postoperative care of patients undergoing endoscopic sinus surgery. *Cochrane Database of Systematic Reviews*. 2018(11).

222. Kennedy DW. Prognostic factors, outcomes and staging in ethmoid sinus surgery. *The Laryngoscope*. 1992;102(12 Pt 2 Suppl 57):1-18.
223. Kuhn FA, Citardi MJ. Advances in postoperative care following functional endoscopic sinus surgery. *Otolaryngologic clinics of North America*. 1997;30(3):479-490.
224. Bugten V, Nordgård S, Skogvoll E, Steinsvåg S. Effects of nonabsorbable packing in middle meatus after sinus surgery. *The laryngoscope*. 2006;116(1):83-88.
225. Rudmik L, Soler ZM, Orlandi RR, et al. Early postoperative care following endoscopic sinus surgery: an evidence - based review with recommendations. In. *International forum of allergy & rhinology*. Vol 1: Wiley Online Library; 2011:417-430.
226. Lee JY, Byun JY. Relationship between the frequency of postoperative debridement and patient discomfort, healing period, surgical outcomes, and compliance after endoscopic sinus surgery. *The Laryngoscope*. 2008;118(10):1868-1872.
227. Kühnel T, Hosemann W, Wagner W, Fayad K. How traumatising is mechanical mucous membrane care after interventions on paranasal sinuses? A histological immunohistochemical study. *Laryngo-rhino-otologie*. 1996;75(10):575-579.
228. Kemppainen T, Seppä J, Tuomilehto H, Kokki H, Nuutinen J. Repeated early debridement does not provide significant symptomatic benefit after ESS. *Rhinology*. 2008;46(3):238-242.
229. Watkinson JCE, Clarke, R. W. (Ed.). (2018). Scott-Brown's Otorhinolaryngology and Head and Neck Surgery. Boca Raton: CRC Press, <https://doi.org/10.1201/9780203731031>.
230. Saleh AM, Torres KM, Murad MH, Erwin PJ, Driscoll CLW. Prophylactic Perioperative Antibiotic Use in Endoscopic Sinus Surgery: A Systematic Review and Meta-analysis. *Otolaryngology–Head and Neck Surgery*. 2012;146(4):533-538.
231. Gouzos M, Ramezanpour M, Bassiouni A, Psaltis AJ, Wormald PJ, Vreugde S. Antibiotics Affect ROS Production and Fibroblast Migration in an In-vitro Model of Sinonasal Wound Healing. *Frontiers in cellular and infection microbiology*. 2020;10:110.
232. Sachse F, von Eiff C, Becker K, Rudack C. Anti-inflammatory effects of ciprofloxacin in *S. aureus* Newman induced nasal inflammation in vitro. *Journal of Inflammation*. 2008;5(1):11.
233. Serra R, Gallelli L, Buffone G, et al. Doxycycline speeds up healing of chronic venous ulcers. *Int Wound J*. 2015;12(2):179-184.
234. Rowe-Jones JM, Medcalf M, Durham SR, Richards DH, Mackay IS. Functional endoscopic sinus surgery: 5 year follow up and results of a prospective, randomised,

- stratified, double-blind, placebo controlled study of postoperative fluticasone propionate aqueous nasal spray. *Rhinology*. 2005;43(1):2-10.
235. Jiang R-S, Liang K-L, Yang K-Y, et al. Postoperative antibiotic care after functional endoscopic sinus surgery. *American journal of rhinology*. 2008;22(6):608-612.
236. Pundir V, Pundir J, Lancaster GA, et al. Role of corticosteroids in functional endoscopic sinus surgery-a systematic review and meta-analysis. *Rhinology*. 2016;54(1):3-19.
237. Howard BE, Lal D. Oral steroid therapy in chronic rhinosinusitis with and without nasal polyposis. *Current allergy and asthma reports*. 2013;13(2):236-243.
238. DelGaudio JM, Wise SK. Topical steroid drops for the treatment of sinus ostia stenosis in the postoperative period. *American journal of rhinology*. 2006;20(6):563-567.
239. Jorissen M, Bachert C. Effect of corticosteroids on wound healing after endoscopic sinus surgery. *Rhinology*. 2009;47(3):280.
240. Bhalla RK, Payton K, Wright ED. Safety of budesonide in saline sinonasal irrigations in the management of chronic rhinosinusitis with polyposis: lack of significant adrenal suppression. *Journal of Otolaryngology-Head and Neck Surgery*. 2008;37(6):821.
241. Welch KC, Thaler ER, Doghramji LL, Palmer JN, Chiu AG. The effects of serum and urinary cortisol levels of topical intranasal irrigations with budesonide added to saline in patients with recurrent polyposis after endoscopic sinus surgery. *American journal of rhinology & allergy*. 2010;24(1):26-28.
242. Illum L. Nasal delivery. The use of animal models to predict performance in man. *Journal of drug targeting*. 1996;3(6):427-442.
243. Jacob A, Faddis BT, Chole RA. Chronic bacterial rhinosinusitis: description of a mouse model. *Archives of Otolaryngology-Head & Neck Surgery*. 2001;127(6):657-664.
244. Valentine R, Jervis-Bardy J, Psaltis A, Tan LW, Wormald PJ. Efficacy of using a hydrodebrider and of citric acid/zwitterionic surfactant on a *Staphylococcus aureus* bacterial biofilm in the sheep model of rhinosinusitis. *Am J Rhinol Allergy*. 2011;25(5):323-326.
245. Singhal D, Jekle A, Debabov D, et al. Efficacy of NVC-422 against *Staphylococcus aureus* biofilms in a sheep biofilm model of sinusitis. *Int Forum Allergy Rhinol*. 2012;2(4):309-315.
246. Thomas DC, Wormald PJ. Standardization of diagnostic criteria for eosinophilic chronic rhinosinusitis in the *Oestrus ovis* infected sheep model. *Am J Rhinol*. 2007;21(5):551-555.

247. Singhal D, Boase S, Field J, Jardeleza C, Foreman A, Wormald PJ. Quantitative analysis of in vivo mucosal bacterial biofilms. *Int Forum Allergy Rhinol.* 2012;2(1):57-62.
248. PJ TLaW. The efficacy of topical antibiofilm agents in a sheep model of rhinosinusitis. *Am J Rhinol.* 2008;22(6)(2008 Nov-Dec;):560-567.
249. Ha KR, Psaltis AJ, Tan L, Wormald PJ. A sheep model for the study of biofilms in rhinosinusitis. *American journal of rhinology.* 2007;21(3):339-345.
250. McIntosh D, Cowin A, Adams D, Wormald P-J. The effect of an expandable polyvinyl acetate (Merocel) pack on the healing of the nasal mucosa of sheep. *American journal of rhinology.* 2005;19(6):577-581.
251. Rajapaksa S, McIntosh D, Cowin A, Adams D, Wormald PJ. The effect of insulin-like growth factor 1 incorporated into a hyaluronic acid-based nasal pack on nasal mucosal healing in a healthy sheep model and a sheep model of chronic sinusitis. *Am J Rhinol.* 2005;19(3):251-256.
252. Athanasiadis T, Beule AG, Robinson BH, Robinson SR, Shi Z, Wormald PJ. Effects of a novel chitosan gel on mucosal wound healing following endoscopic sinus surgery in a sheep model of chronic rhinosinusitis. *The Laryngoscope.* 2008;118(6):1088-1094.
253. Robinson S, Adams D, Wormald PJ. The effect of nasal packing and prednisolone on mucosal healing and reciliation in a sheep model. *Rhinology.* 2004;42(2):68-72.
254. Liang KL, Jiang RS, Wang J, et al. Developing a rabbit model of rhinogenic chronic rhinosinusitis. *The Laryngoscope.* 2008;118(6):1076-1081.
255. Miller RS, Steward DL, Tami TA, et al. The clinical effects of hyaluronic acid ester nasal dressing (Merogel) on intranasal wound healing after functional endoscopic sinus surgery. *Otolaryngology—Head and Neck Surgery.* 2003;128(6):862-869.
256. Vaiman M, Eviatar E, Segal S. Effectiveness of second-generation fibrin glue in endonasal operations. *Otolaryngology—Head and Neck Surgery.* 2002;126(4):388-391.
257. Pomerantz J, Dutton JM. Platelet gel for endoscopic sinus surgery. *Annals of Otology, Rhinology & Laryngology.* 2005;114(9):699-704.
258. Franklin JH, Wright ED. Randomized, controlled, study of absorbable nasal packing on outcomes of surgical treatment of rhinosinusitis with polyposis. *American journal of rhinology.* 2007;21(2):214-217.
259. Wormald PJ, Boustred RN, Le T, Hawke L, Sacks R. A prospective single-blind randomized controlled study of use of hyaluronic acid nasal packs in patients after endoscopic sinus surgery. *Am J Rhinol.* 2006;20(1):7-10.

260. Chandra RK, Conley DB, Haines III GK, Kern RC. Long-term effects of FloSeal™ packing after endoscopic sinus surgery. *American journal of rhinology*. 2005;19(3):240-243.
261. Karkos P, Thinakararajan T, Goodyear P, Srinivasan V. Day-case endoscopic sinus surgery using dissolvable haemostatic nasal packs: a pilot study. *European archives of oto-rhino-laryngology*. 2007;264(10):1171-1174.
262. Kastl KG, Betz CS, Siedek V, Leunig A. Control of bleeding following functional endoscopic sinus surgery using carboxy-methylated cellulose packing. *European archives of oto-rhino-laryngology*. 2009;266(8):1239-1243.
263. Catalano PJ, Roffman EJ. Evaluation of middle meatal stenting after minimally invasive sinus techniques (MIST). *Otolaryngology - Head and Neck Surgery*. 2003;128(6):875-881.
264. Tom LW, Palasti S, Potsic WP, Handler SD, Wetmore RF. The effects of gelatin film stents in the middle meatus. *American journal of rhinology*. 1997;11(3):229-232.
265. Kao S, Liao C, Tseng J. Dacryocystorhinostomy with intraoperative mitomycin C. *Ophthalmol* 1997; 104: 86-91. In.
266. Chung JH, Cosenza MJ, Rahbar R, Metson RB. Mitomycin C for the prevention of adhesion formation after endoscopic sinus surgery: a randomized, controlled study. *Otolaryngology—Head and Neck Surgery*. 2002;126(5):468-474.
267. Anand VK, Tabaei A, Kacker A, Newman JG, Huang C. The role of mitomycin C in preventing synechia and stenosis after endoscopic sinus surgery. *American journal of rhinology*. 2004;18(5):311-314.
268. Chan K-O, Gervais M, Tsaparas Y, Genoway KA, Manarey C, Javer AR. Effectiveness of intraoperative mitomycin C in maintaining the patency of a frontal sinusotomy: a preliminary report of a double-blind randomized placebo-controlled trial. *American journal of rhinology*. 2006;20(3):295-299.
269. Valentine R, Wormald PJ. Are routine dissolvable nasal dressings necessary following endoscopic sinus surgery? *Laryngoscope*. 2010;120(10):1920-1921.
270. Valentine R, Wormald P-J, Sindwani R. Advances in absorbable biomaterials and nasal packing. *Otolaryngologic Clinics of North America*. 2009;42(5):813-828.
271. Menzies D, Ellis H. Intestinal obstruction from adhesions--how big is the problem? *Ann R Coll Surg Engl*. 1990;72(1):60-63.
272. ten Broek RPG, Issa Y, van Santbrink EJP, et al. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. *BMJ : British Medical Journal*. 2013;347:f5588.
273. Correa-Rovelo JM, Cleva Villanueva-López G, Medina-Santillan R, Carrillo-Esper R, Díaz-Girón-Gidi A. Intestinal obstruction secondary to postoperative adhesion

- formation in abdominal surgery. Review literature. *Cirugía y Cirujanos (English Edition)*. 2015;83(4):345-351.
274. Ray NF, Denton WG, Thamer M, Henderson SC, Perry S. Abdominal adhesiolysis: inpatient care and expenditures in the United States in 1994. *Journal of the American College of Surgeons*. 1998;186(1):1-9.
275. Muller GP, Rademaker LA. Role of infection in the production of postoperative adhesions. *Archives of surgery*. 1933;26(2):280-287.
276. Edna T-H, Talabani AJ, Lydersen S, Endreseth BH. Survival after acute colon diverticulitis treated in hospital. *International journal of colorectal disease*. 2014;29(11):1361-1367.
277. Han EC, Ryoo S-B, Park BK, et al. Surgical outcomes and prognostic factors of emergency surgery for colonic perforation: would fecal contamination increase morbidity and mortality? *International journal of colorectal disease*. 2015;30(11):1495-1504.
278. diZerega GSe, SpringerLink. *Peritoneal Surgery*. Springer New York : Imprint: Springer; 2000.
279. Ellis H, Harrison W, Hugh T. The healing of peritoneum under normal and pathological conditions. *British journal of Surgery*. 1965;52(6):471-476.
280. Watters W, WB W, RC B. Scanning electron microscopy of mesothelial regeneration in the rat. 1972.
281. Raftery A. Regeneration of peritoneum: a fibrinolytic study. *J Anat*. 1979;129(Pt 3):659.
282. Johnson BZ, Stevenson AW, Prêle CM, Fear MW, Wood FM. The Role of IL-6 in Skin Fibrosis and Cutaneous Wound Healing. *Biomedicines*. 2020;8(5):101.
283. Cheong Y, Shelton J, Laird S, et al. IL-1, IL-6 and TNF- $\alpha$  concentrations in the peritoneal fluid of women with pelvic adhesions. *Human Reproduction*. 2002;17(1):69-75.
284. Hunter CA, Jones SA. IL-6 as a keystone cytokine in health and disease. *Nature immunology*. 2015;16(5):448-457.
285. Collignon P, Nimmo GR, Gottlieb T, Gosbell IB. *Staphylococcus aureus* Bacteremia, Australia. *Emerging Infectious Disease journal*. 2005;11(4):554.
286. Shogan BD, Carlisle EM, Alverdy JC, Umanskiy K. Do we really know why colorectal anastomoses leak? *Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract*. 2013;17(9):1698-1707.

287. Herzog T, Chromik AM, Uhl W. Treatment of complicated intra-abdominal infections in the era of multi-drug resistant bacteria. *European journal of medical research*. 2010;15(12):525-532.
288. Okabayashi K, Ashrafian H, Zacharakis E, et al. Adhesions after abdominal surgery: a systematic review of the incidence, distribution and severity. *Surgery today*. 2014;44(3):405-420.
289. Pessaux P, Msika S, Atalla D, Hay JM, Flamant Y, French Association for Surgical R. Risk factors for postoperative infectious complications in noncolorectal abdominal surgery: a multivariate analysis based on a prospective multicenter study of 4718 patients. *Archives of surgery*. 2003;138(3):314-324.
290. Montravers P, Lepape A, Dubreuil L, et al. Clinical and microbiological profiles of community-acquired and nosocomial intra-abdominal infections: results of the French prospective, observational EBIIA study. *The Journal of antimicrobial chemotherapy*. 2009;63(4):785-794.
291. Egea AM, Albasini JA, Carmona GZ, Paricio PP. Adhesion response to different forms of treating a peritoneal lesion: an experimental study in rats. *Digestive surgery*. 1995;12(6):334-337.
292. Ling F, Stovall T, Meyer N, Elkins T, Muram D. Adhesion formation associated with the use of absorbable staples in comparison to other types of peritoneal injury. *International Journal of Gynecology & Obstetrics*. 1989;30(4):361-366.
293. Ergul E, Korukluoglu B. Peritoneal adhesions: facing the enemy. *International journal of surgery*. 2008;6(3):253-260.
294. Vrijland W, Jeekel J, Van Geldorp H, Swank D, Bonjer H. Abdominal adhesions: intestinal obstruction, pain, and infertility. *Surgical Endoscopy and Other Interventional Techniques*. 2003;17(7):1017-1022.
295. Hodgkin T. Lectures on the Morbid Anatomy of the Serous and Mucous Membranes, 2, 368. In: London; 1840.
296. Gibson CL. VI. A Study of One Thousand Operations for Acute Intestinal Obstruction and Gangrenous Hernia (Concluded.). *Annals of surgery*. 1900;32(5):676.
297. Soybel D, Landman WB. Ileus and bowel obstruction. In: *Greenfield's Surgery: Scientific Principles and Practice: Fifth Edition*. Wolters Kluwer Health Adis (ESP); 2012:749-772.
298. Krielen P, Stommel MW, Pargmae P, et al. Adhesion-related readmissions after open and laparoscopic surgery: a retrospective cohort study (SCAR update). *The Lancet*. 2020;395(10217):33-41.
299. Sebbag L, Even M, Fay S, et al. Early Second-Look Hysteroscopy: Prevention and Treatment of Intrauterine Post-surgical Adhesions. *Frontiers in Surgery*. 2019;6(50).

300. ten Broek RP, Strik C, Issa Y, Bleichrodt RP, van Goor H. Adhesiolysis-related morbidity in abdominal surgery. *Annals of surgery*. 2013;258(1):98-106.
301. Eliezer DD, Holmes M, Sullivan G, et al. High-Risk Emergency Laparotomy in Australia: Comparing NELA, P-POSSUM, and ACS-NSQIP Calculators. *Journal of Surgical Research*. 2020;246:300-304.
302. Sikirica V, Bapat B, Candrilli SD, Davis KL, Wilson M, Johns A. The inpatient burden of abdominal and gynecological adhesiolysis in the US. *BMC Surg*. 2011;11:13.
303. Ryan GB, Grobéty J, Majno G. Postoperative peritoneal adhesions: a study of the mechanisms. *The American journal of pathology*. 1971;65(1):117.
304. Połubinska A, Winckiewicz M, Staniszewski R, Bręborowicz A, Oreopoulos D. Time to reconsider saline as the ideal rinsing solution during abdominal surgery. *The American journal of surgery*. 2006;192(3):281-285.
305. Mais V. Peritoneal adhesions after laparoscopic gastrointestinal surgery. *World journal of gastroenterology*. 2014;20(17):4917-4925.
306. Lauder CI, Garcea G, Strickland A, Maddern GJ. Abdominal adhesion prevention: still a sticky subject? *Digestive surgery*. 2010;27(5):347-358.
307. Perlitz Y, Oettinger M, Karam K, Lipshitz B, Simon K. Anaphylactic shock during hysteroscopy using Hyskon solution: case report and review of adverse reactions and their treatment. *Gynecologic and obstetric investigation*. 1996;41(1):67-69.
308. Beck DE, Cohen Z, Fleshman JW, et al. A prospective, randomized, multicenter, controlled study of the safety of Seprafilm adhesion barrier in abdominopelvic surgery of the intestine. *Diseases of the colon and rectum*. 2003;46(10):1310-1319.
309. Dörr P, Vemer H, Brommer E, Willemsen W, Veldhuizen R, Rolland R. Prevention of postoperative adhesions by tissue-type plasminogen activator (t-PA) in the rabbit. *European Journal of Obstetrics & Gynecology*. 1990;37(3):287-291.
310. Montz F, Fowler JM, Wolff AJ, Lacey SM, Mohler M. The ability of recombinant tissue plasminogen activator to inhibit post-radical pelvic surgery adhesions in the dog model. *American journal of obstetrics and gynecology*. 1991;165(5):1539-1542.
311. Legrand EK, Rodgers KE, Girgis W, Campeau JD, Dizerega GS. Comparative efficacy of nonsteroidal anti-inflammatory drugs and anti-thromboxane agents in a rabbit adhesion-prevention model. *Journal of Investigative Surgery*. 1995;8(3):187-194.
312. Rodgers KE, Girgis W, Campeau JD, Dizerega GS. Reduction of adhesion formation by intraperitoneal administration of a recombinant Hirudin analog. *Journal of Investigative Surgery*. 1996;9(5):385-391.



313. Jackson JK, Skinner KC, Burgess L, Sun T, Hunter WL, Burt HM. Paclitaxel-loaded crosslinked hyaluronic acid films for the prevention of postsurgical adhesions. *Pharmaceutical research*. 2002;19(4):411-417.
314. Cashman J, Burt H, Springate C, Gleave J, Jackson J. Camptothecin-loaded films for the prevention of postsurgical adhesions. *Inflammation Research*. 2004;53(8):355-362.
315. Nehéz L, Vödrös D, Axelsson J, Tingstedt B, Lindman B, Andersson R. Prevention of postoperative peritoneal adhesions: effects of lysozyme, polylysine and polyglutamate versus hyaluronic acid. *Scandinavian journal of gastroenterology*. 2005;40(9):1118-1123.
316. Fukasawa M, Girgis W. Inhibition of postsurgical adhesions in a standardized rabbit model: II. Intraperitoneal treatment with heparin. *International journal of fertility*. 1991;36(5):296-301.
317. Tayyar M, Turan R, Ayata D. The use of amniotic membrane plus heparin to prevent postoperative adhesions in the rabbit. *The Tokai journal of experimental and clinical medicine*. 1993;18(1-2):57-60.
318. Arikan S, Adas G, Barut G, et al. An evaluation of low molecular weight heparin and hyperbaric oxygen treatment in the prevention of intra-abdominal adhesions and wound healing. *The American journal of surgery*. 2005;189(2):155-160.
319. Muzii L, Marana R, Brunetti L, Margutti F, Vacca M, Mancuso S. Postoperative adhesion prevention with low-dose aspirin: effect through the selective inhibition of thromboxane production. *Human reproduction (Oxford, England)*. 1998;13(6):1486-1489.
320. Rodgers KE, Girgis W, Campeau J, Dizerega GS. Reduction of adhesion formation by intraperitoneal administration of Aro-Gly-Asp-containing peptides. *Fertility and Sterility*. 1998;70(6):1131-1138.
321. Sahin M, Cakir M, Avsar FM, Tekin A, Kucukkartallar T, Akoz M. The effects of anti-adhesion materials in preventing postoperative adhesion in abdominal cavity (anti-adhesion materials for postoperative adhesions). *Inflammation*. 2007;30(6):244-249.
322. Höckel M, Ott S, Siemann U, Kissel T. Prevention of peritoneal adhesions in the rat with sustained intraperitoneal dexamethasone delivered by a novel therapeutic system. In. *Annales chirurgiae et gynaecologiae*. Vol 761987:306-313.
323. Montz F, Monk BJ, Lacy SM, Fowler JM. Ketorolac tromethamine, a nonsteroidal anti-inflammatory drug: ability to inhibit post-radical pelvic surgery adhesions in a porcine model. *Gynecologic oncology*. 1993;48(1):76-79.
324. Silverman S, Ambrose N, Youngs D, Shepherd A, Roberts A, Keighley M. The effect of peritoneal lavage with tetracycline solution on postoperative infection. *Diseases of the colon & rectum*. 1986;29(3):165-169.

325. Weinans MJ, Kauer FM, Klompmaker IJ, Wijma J. Transient liver function disturbances after the intraperitoneal use of 32% dextran 70 as adhesion prophylaxis in infertility surgery. *Fertility and sterility*. 1990;53(1):159-161.
326. Diamond MP, DeCherney A, Linsky C, Cunningham T, Constantine B. Assessment of carboxymethylcellulose and 32% dextran 70 for prevention of adhesions in a rabbit uterine horn model. *International journal of fertility*. 1988;33(4):278-282.
327. Sagliyan A, Gunay C, Han MC, Sakin F, Hayat A. An experimental study on the efficacy of sodium hyaluronate in prevention of postoperative intraperitoneal adhesions. *J Anim Vet Adv*. 2009;8(4):664-668.
328. Burns JW, Skinner K, Colt J, et al. Prevention of tissue injury and postsurgical adhesions by precoating tissues with hyaluronic acid solutions. *Journal of Surgical Research*. 1995;59(6):644-652.
329. Johns DB, Rodgers KE, Donahue WD, Kiorpes TC. Reduction of adhesion formation by postoperative administration of ionically cross-linked hyaluronic acid. *Fertility and sterility*. 1997;68(1):37-42.
330. De Iaco PA, Muzzupapa G, Bigon E, et al. Efficacy of a hyaluronan derivative gel in postsurgical adhesion prevention in the presence of inadequate hemostasis. *Surgery*. 2001;130(1):60-64.
331. Mais V, Bracco G, Litta P, Gargiulo T, Melis GB. Reduction of postoperative adhesions with an auto-crosslinked hyaluronan gel in gynaecological laparoscopic surgery: a blinded, controlled, randomized, multicentre study. *Human Reproduction*. 2006;21(5):1248-1254.
332. Lauder CI, Garcea G, Strickland A, Maddern GJ. Use of a modified chitosan-dextran gel to prevent peritoneal adhesions in a rat model. *The Journal of surgical research*. 2011;171(2):877-882.
333. Arora M, Jaroudi KA, Hamilton CJ, Dayel F. Controlled comparison of interceed and amniotic membrane graft in the prevention of postoperative adhesions in the rabbit uterine horn model. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 1994;55(3):179-182.
334. Wallwiener D, Meyer A, Bastert G. Adhesion formation of the parietal and visceral peritoneum: an explanation for the controversy on the use of autologous and alloplastic barriers? *Fertility and sterility*. 1998;69(1):132-137.
335. Karabulut B, Sönmez K, Türkyılmaz Z, et al. Omentum prevents intestinal adhesions to mesh graft in abdominal infections and serosal defects. *Surgical Endoscopy And Other Interventional Techniques*. 2006;20(6):978-982.
336. Becker JM, Dayton MT, Fazio VW, et al. Prevention of postoperative abdominal adhesions by a sodium hyaluronate-based bioresorbable membrane: a prospective,

- randomized, double-blind multicenter study. *Journal of the American College of Surgeons*. 1996;183(4):297-306.
337. Mettler L. Adhibit adhesion prevention gel myomectomy study. In. *19th Annual European Congress of Obstetrics and Gynecology*. Turin2006.
338. Di Zerega G, Verco S, Young P, et al. A randomized, controlled pilot study of the safety and efficacy of 4% icodextrin solution in the reduction of adhesions following laparoscopic gynaecological surgery. *Human Reproduction*. 2002;17(4):1031-1038.
339. Mettler L, Audebert A, Lehmann-Willenbrock E, Schive-Peterhansl K, Jacobs VR. A randomized, prospective, controlled, multicenter clinical trial of a sprayable, site-specific adhesion barrier system in patients undergoing myomectomy. *Fertility and sterility*. 2004;82(2):398-404.
340. Rice VM, Shanti A, Moghissi KS, Leach RE. A comparative evaluation of Poloxamer 407 and oxidized regenerated cellulose (Interceed [TC7]) to reduce postoperative adhesion formation in the rat uterine horn model. *Fertility and sterility*. 1993;59(4):901-906.
341. March CM, Boyers S, Franklin R, et al. Prevention of adhesion formation/reformation with the Gore-Tex Surgical Membrane. *Progress in clinical and biological research*. 1993;381:253.
342. Martín-Cartes JA, Morales-Conde S, Suárez-Grau JM, et al. Role of fibrin glue in the prevention of peritoneal adhesions in ventral hernia repair. *Surgery today*. 2008;38(2):135-140.
343. Raftery AT. Absorbable haemostatic materials and intraperitoneal adhesion formation. *British Journal of Surgery*. 1980;67(1):57-58.
344. Reid RL, Hahn PM, Spence JE, Tulandi T, Yuzpe AA, Wiseman DM. A randomized clinical trial of oxidized regenerated cellulose adhesion barrier (Interceed, TC7) alone or in combination with heparin. *Fertility and sterility*. 1997;67(1):23-29.
345. Wiseman D, Kamp L, Saferstein L, Linsky C, Gottlick L, Diamond MP. Improving the efficacy of INTERCEED Barrier in the presence of blood using thrombin, heparin or a blood insensitive barrier, modified INTERCEED (nTC7). *Progress in clinical and biological research*. 1993;381:205-212.
346. Oh SH, Kim JK, Song KS, et al. Prevention of postsurgical tissue adhesion by anti - inflammatory drug - loaded pluronic mixtures with sol-gel transition behavior. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*. 2005;72(3):306-316.
347. Shapiro I, Granat M, Sharf M. The effect of intraperitoneal colchicine on the formation of peritoneal adhesions in the rat. *Archives of gynecology*. 1982;231(3):227-233.

348. Blauer KL, Collins RL. The effect of intraperitoneal progesterone on postoperative adhesion formation in rabbits. *Fertility and sterility*. 1988;49(1):144-149.
349. Steinleitner A, Lambert H, Kazensky C, Sanchez I, Sueldo C. Reduction of primary postoperative adhesion formation under calcium channel blockade in the rabbit. *Journal of Surgical Research*. 1990;48(1):42-45.
350. Ar'Rajab A, Snoj M, Larsson K, Bengmark S. Exogenous phospholipid reduces postoperative peritoneal adhesions in rats. *The European Journal of Surgery= Acta Chirurgica*. 1995;161(5):341-344.
351. de la Portilla F, Ynfante I, Bejarano D, et al. Prevention of peritoneal adhesions by intraperitoneal administration of vitamin E: an experimental study in rats. *Diseases of the colon & rectum*. 2004;47(12):2157-2161.
352. Ghahiri A-A, Zarean E, Rasti M, ADIBI S. Effect of D-penicillamine in prevention of post operative pelvic adhesion formation in rat. 2006.
353. Dinc S, Ozaslan C, Kuru B, et al. Methylene blue prevents surgery-induced peritoneal adhesions but impairs the early phase of anastomotic wound healing. *Canadian journal of surgery*. 2006;49(5):321.
354. van der Wal JB, Jeekel J. The use of statins in postoperative adhesion prevention. *Annals of surgery*. 2007;245(2):185.
355. Uguralp S, Akin M, Karabulut AB, et al. Reduction of peritoneal adhesions by sustained and local administration of epidermal growth factor. *Pediatric surgery international*. 2008;24(2):191-197.
356. Mais V, Ajossa S, Piras B, Guerriero S, Marongiu D, Melis GB. Prevention of de-novo adhesion formation after laparoscopic myomectomy: a randomized trial to evaluate the effectiveness of an oxidized regenerated cellulose absorbable barrier. *Human reproduction*. 1995;10(12):3133-3135.
357. Lee W-S, Baek JH, Lee WK. Direct comparison of Seprafilm® versus Adept® versus no additive for reducing the risk of small-bowel obstruction in colorectal cancer surgery. *Surgery today*. 2013;43(9):995-1002.
358. Tang C-L, Jayne DG, Seow-Choen F, Ng Y-Y, Eu K-W, Mustapha N. A randomized controlled trial of 0.5% ferric hyaluronate gel (Intergel) in the prevention of adhesions following abdominal surgery. *Annals of surgery*. 2006;243(4):449.
359. Mayes SM, Davis J, Scott J, et al. Polysaccharide-based films for the prevention of unwanted postoperative adhesions at biological interfaces. *Acta Biomaterialia*. 2020.
360. Lee CH, Kim H, Han IW, et al. Effect of polylactic film (Surgi-Wrap) on preventing postoperative ileus after major hepato-pancreato-biliary surgery. *Annals of Hepato-biliary-pancreatic Surgery*. 2016;20(4):191-196.

361. Hu C, Tang F, Wu Q, et al. Novel trilaminar polymeric anti-adhesion membrane prevents postoperative pericardial adhesion. *The Annals of Thoracic Surgery*. 2020.
362. Lauder CI, Strickland A, Maddern GJ. Use of a modified chitosan-dextran gel to prevent peritoneal adhesions in a porcine hemicolectomy model. *J Surg Res*. 2012;176(2):448-454.
363. Tzianabos AO, Cisneros RL, Gershkovich J, et al. Effect of surgical adhesion reduction devices on the propagation of experimental intra-abdominal infection. *Archives of surgery*. 1999;134(11):1254-1259.
364. Reijnen MM, Meis JF, Postma VA, Van Goor H. Prevention of intra-abdominal abscesses and adhesions using a hyaluronic acid solution in a rat peritonitis model. *Archives of surgery*. 1999;134(9):997-1001.
365. Richter K, Thomas N, Claeys J, et al. A Topical Hydrogel with Deferiprone and Gallium-Protoporphyrin Targets Bacterial Iron Metabolism and Has Antibiofilm Activity. *Antimicrob Agents Chemother*. 2017;61(6).
366. Richter K, Thomas N, Zhang G, et al. Deferiprone and Gallium-Protoporphyrin Have the Capacity to Potentiate the Activity of Antibiotics in Staphylococcus aureus Small Colony Variants. *Front Cell Infect Microbiol*. 2017;7:280.
367. VEDIAPPAN RS, Mascarenhas A, Nguyen-Hoang A, et al. Prevention of peridural adhesions in spinal surgery: Assessing safety and efficacy of Chitogel with Deferiprone in a sheep model. *Journal of Clinical Neuroscience*. 2020;72:378-385.
368. Wiseman DM. Animal Adhesion Models: Design, Variables, and Relevance. In: diZerega GS, ed. *Peritoneal Surgery*. New York, NY: Springer New York; 2000:459-476.
369. Tang J, Xiang Z, Bernards MT, Chen S. Peritoneal adhesions: Occurrence, prevention and experimental models. *Acta Biomaterialia*. 2020.
370. Kaplun A, Griffel B, Halperin B, Aronson M. A model for adhesion formation by thermal injury in the abdominal cavity of the mouse. *European surgical research*. 1984;16(2):131-135.
371. Rappaport WD, Holcomb M, Valente J, Chvapil M. Antibiotic irrigation and the formation of intraabdominal adhesions. *The American journal of surgery*. 1989;158(5):435-437.
372. Lehman G, Filipi CJ. Method of promoting tissue adhesion. In: Google Patents; 2012.
373. Linsky C, Diamond M, DeCherney A, DiZerega G, Cunningham T. Effect of blood on the efficacy of barrier adhesion reduction in the rabbit uterine horn model. *Infertility*. 1988;11(4):273-280.

374. Hoban LD, Pierce M, Quance J, et al. Use of polypentapeptides of elastin to prevent postoperative adhesions: efficacy in a contaminated peritoneal model. *Journal of surgical research*. 1994;56(2):179-183.
375. Hartvigsen J, Hancock MJ, Kongsted A, et al. What low back pain is and why we need to pay attention. *Lancet*. 2018;391(10137):2356-2367.
376. Chan C-W, Peng P. Review Article: Failed Back Surgery Syndrome. *Pain Medicine*. 2011;12(577-606).
377. Rhyne AL, Blumenthal SL, Frank EH, et al. Oxiplex reduces leg pain, back pain, and associated symptoms after lumbar discectomy. *Spine (Phila Pa 1976)*. 2012;37(8):631-641.
378. Selkirk SM, Ruff R. Chapter 53 - Low back pain, radiculopathy. In: Masdeu JC, González RG, eds. *Handbook of Clinical Neurology*. Vol 136. Elsevier; 2016:1027-1033.
379. Guyer RD, Patterson M, Ohnmeiss DD. Failed back surgery syndrome: diagnostic evaluation. *J Am Acad Orthop Surg*. 2006;14(9):534-543.
380. Harris IA, Dao AT. Trends of spinal fusion surgery in Australia: 1997 to 2006. *ANZ J Surg*. 2009;79(11):783-788.
381. Foster NE, Anema JR, Cherkin D, et al. Prevention and treatment of low back pain: evidence, challenges, and promising directions. *Lancet*. 2018;391(10137):2368-2383.
382. Thomson S. Failed back surgery syndrome - definition, epidemiology and demographics. *Br J Pain*. 2013;7(1):56-59.
383. Bosscher HA, Heavner JE. Incidence and severity of epidural fibrosis after back surgery: an endoscopic study. *Pain Pract*. 2010;10(1):18-24.
384. Baber Z, Erdek MA. Failed back surgery syndrome: current perspectives. *J Pain Res*. 2016;9:979-987.
385. Parker SL, Mendenhall SK, Godil SS, et al. Incidence of Low Back Pain After Lumbar Discectomy for Herniated Disc and Its Effect on Patient-reported Outcomes. *Clin Orthop Relat Res*. 2015;473(6):1988-1999.
386. Foster NE, Underwood M, Maher CG, Hartvigsen J, van Tulder M, Buchbinder R. Low back pain - Authors' reply. *Lancet*. 2018;392(10164):2549-2550.
387. Wang H, Sun W, Fu D, Shen Y, Chen YY, Wang LL. Update on biomaterials for prevention of epidural adhesion after lumbar laminectomy. *J Orthop Translat*. 2018;13:41-49.
388. Cho YJ, Lee CH, Kim DW, et al. Effects of silk solution against laminectomy-induced dural adhesion formation and inflammation in a rat model. *J Neurosurg Spine*. 2018;29(5):599-607.

389. Key J FL. Experimental intervertebral disc lesions. *J Bone Joint Surg Am*. 1948;;30(3):621e30.
390. Songer MN, Ghosh L, Spencer DL. Effects of sodium hyaluronate on peridural fibrosis after lumbar laminotomy and discectomy. *Spine (Phila Pa 1976)*. 1990;15(6):550-554.
391. Mohi Eldin MM, Abdel Razek NM. Epidural Fibrosis after Lumbar Disc Surgery: Prevention and Outcome Evaluation. *Asian Spine J*. 2015;9(3):370-385.
392. Babar S, Saifuddin A. MRI of the post-discectomy lumbar spine. *Clinical radiology*. 2002;57(11):969-981.
393. Delitto A, Piva SR, Moore CG, et al. Surgery versus nonsurgical treatment of lumbar spinal stenosis: a randomized trial. *Annals of internal medicine*. 2015;162(7):465-473.
394. Khosravi M, Azemati S, Sahmeddini M. Gabapentin versus naproxen in the management of failed back surgery syndrome; a randomized controlled trial. *Acta Anaesthesiol Belg*. 2014;65(1):31-37.
395. Franklin GM. Opioids for chronic noncancer pain: a position paper of the American Academy of Neurology. *Neurology*. 2014;83(14):1277-1284.
396. Bicket MC, Horowitz JM, Benzon HT, Cohen SP. Epidural injections in prevention of surgery for spinal pain: systematic review and meta-analysis of randomized controlled trials. *The Spine Journal*. 2015;15(2):348-362.
397. Manchikanti L. The growth of interventional pain management in the new millennium: A critical analysis of utilization in the Medicare population. *Pain Physician*. 2004;7:465-482.
398. Hiremath GK, Perez-Cruet MJ. Chapter 153 - Minimally Invasive Posterior Cervical Foraminotomy and Microdiscectomy. In: Quiñones-Hinojosa A, ed. *Schmidek and Sweet Operative Neurosurgical Techniques (Sixth Edition)*. Philadelphia: W.B. Saunders; 2012:1771-1776.
399. Wu P-F, Liu B-H, Wang B, et al. Complications of Full-Endoscopic Versus Microendoscopic Foraminotomy for Cervical Radiculopathy: A Systematic Review and Meta-Analysis. *World neurosurgery*. 2018;114:217-227.
400. Ross JS, Obuchowski N, Zepp R. The postoperative lumbar spine: evaluation of epidural scar over a 1-year period. *AJNR Am J Neuroradiol*. 1998;19(1):183-186.
401. Sun Y, Wang L-X, Wang L, et al. A comparison of the effectiveness of mitomycin C and 5-fluorouracil in the prevention of peridural adhesion after laminectomy. *Journal of Neurosurgery: Spine*. 2007;7(4):423-428.

402. Fuming T, Changwu D, Songtao Q, Liquan Z, Bo C, Haicheng Y. Preventive effect of dexamethasone gelatin sponge on the lumbosacral epidural adhesion. *Int J Clin Exp Med*. 2015;8(4):5478-5484.
403. Sun Y, Wang L, Sun S, Liu B, Wu N, Cao X. The effect of 10-hydroxycamptothecin in preventing fibroblast proliferation and epidural scar adhesion after laminectomy in rats. *European Journal of Pharmacology*. 2008;593(1-3):44-48.
404. Gürer B, Kahveci R, Gökçe EC, Ozevren H, Turkoglu E, Gökçe A. Evaluation of topical application and systemic administration of rosuvastatin in preventing epidural fibrosis in rats. *The Spine Journal*. 2015;15(3):522-529.
405. Costandi S, Garcia - Jacques M, Dews T, et al. Optimal Temperature for Radiofrequency Ablation of Lumbar Medial Branches for Treatment of Facet - Mediated Back Pain. *Pain Practice*. 2016;16(8):961-968.
406. Zucco F, Ciampichini R, Lavano A, et al. Cost - effectiveness and cost - utility analysis of spinal cord stimulation in patients with failed back surgery syndrome: results from the PRECISE study. *Neuromodulation: Technology at the Neural Interface*. 2015;18(4):266-276.
407. Chen L, Li X-q, Cao L-p, et al. An injectable hydrogel with or without drugs for prevention of epidural scar adhesion after laminectomy in rats. *Chinese Journal of Polymer Science*. 2016;34(2):147-163.
408. Kanamori M, Kawaguchi Y, Ohmori K, Kimura T, Tsuji H, Matsui H. The fate of autogenous free-fat grafts after posterior lumbar surgery: Part 2. Magnetic resonance imaging and histologic studies in repeated surgery cases. *Spine*. 2001;26(20):2264-2270.
409. Kanamori M, Kawaguchi Y, Ohmori K, Kimura T, Tsuji H, Matsui H. The fate of autogenous free-fat grafts after posterior lumbar surgery: part 1. A postoperative serial magnetic resonance imaging study. *Spine*. 2001;26(20):2258-2263.
410. Wang K, long Li X, Liu J, Sun X, Yang H, Gao X. Using cross-linked hyaluronic acid gel to prevent postoperative lumbar epidural space adhesion: in vitro and in vivo studies. *European Spine Journal*. 2020;29(1):129-140.
411. Tao H, Fan H. Implantation of amniotic membrane to reduce postlaminectomy epidural adhesions. *European Spine Journal*. 2009;18(8):1202-1212.
412. Wang Y, Liang M, Zheng Z, et al. Adhesion prevention after laminectomy using silk - polyethylene glycol hydrogels. *Advanced healthcare materials*. 2015;4(14):2120-2127.
413. Rodgers KE, Robertson JT, Espinoza T, et al. Reduction of epidural fibrosis in lumbar surgery with Oxiplex adhesion barriers of carboxymethylcellulose and polyethylene oxide. *Spine J*. 2003;3(4):277-283; discussion 284.
414. <https://clinicaltrials.gov/ct2/show/NCT03433391>.



415. Ong SY, Wu J, Moomhala SM, Tan MH, Lu J. Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. *Biomaterials*. 2008;29(32):4323-4332.
416. Rajiv S, Harding M, Bassiouni A, et al. The efficacy and safety of chitosan dextran gel in a burr hole neurosurgical sheep model. *Acta Neurochir (Wien)*. 2013;155(7):1361-1366; discussion 1366.
417. Rajiv S, Drilling A, Bassiouni A, et al. Chitosan Dextran gel as an anti adhesion agent in a postlaminectomy spinal sheep model. *J Clin Neurosci*. 2017;40:153-156.
418. de Léséleuc L, Harris G, KuoLee R, Chen W. In vitro and in vivo biological activities of iron chelators and gallium nitrate against *Acinetobacter baumannii*. *Antimicrobial agents and chemotherapy*. 2012;56(10):5397-5400.
419. Tricta F, Uetrecht J, Galanello R, et al. Deferiprone-induced agranulocytosis: 20 years of clinical observations. *Am J Hematol*. 2016;91(10):1026-1031.
420. Kandziora F, Pflugmacher R, Scholz M, et al. Comparison between sheep and human cervical spines: an anatomic, radiographic, bone mineral density, and biomechanical study. *Spine*. 2001;26(9):1028-1037.
421. Wilke HJ, Kettler A, Claes LE. Are sheep spines a valid biomechanical model for human spines? *Spine*. 1997;22(20):2365-2374.
422. Sanborn MR, Balzer J, Gerszten PC, Karausky P, Cheng BC, Welch WC. Safety and efficacy of a novel ultrasonic osteotome device in an ovine model. *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia*. 2011;18(11):1528-1533.
423. Wong J, Hemley S, Jones N, Cheng S, Bilston L, Stoodley M. Fluid outflow in a large-animal model of posttraumatic syringomyelia. *Neurosurgery*. 2012;71(2):474-480; discussion 480.
424. Fokkens WJ, Lund VJ, Mullol J, et al. EPOS 2012: European position paper on rhinosinusitis and nasal polyps 2012. A summary for otorhinolaryngologists. *Rhinology*. 2012;50(1):1-12.
425. Lam K, Schleimer R, Kern RC. The Etiology and Pathogenesis of Chronic Rhinosinusitis: a Review of Current Hypotheses. *Curr Allergy Asthma Rep*. 2015;15(7):41.
426. Ahmed A, Earl J, Retchless A, et al. Comparative genomic analyses of 17 clinical isolates of *Gardnerella vaginalis* provide evidence of multiple genetically isolated clades consistent with subspeciation into genovars. *J Bacteriol*. 2012;194(15):3922-3937.
427. Foreman A, Boase S, Psaltis A, Wormald PJ. Role of bacterial and fungal biofilms in chronic rhinosinusitis. *Curr Allergy Asthma Rep*. 2012;12(2):127-135.

428. Singhal D, Psaltis AJ, Foreman A, Wormald PJ. The impact of biofilms on outcomes after endoscopic sinus surgery. *Am J Rhinol Allergy*. 2010;24(3):169-174.
429. Jervis-Bardy J, Boase S, Psaltis A, Foreman A, Wormald PJ. A randomized trial of mupirocin sinonasal rinses versus saline in surgically recalcitrant staphylococcal chronic rhinosinusitis. *Laryngoscope*. 2012;122(10):2148-2153.
430. Ooi ML, Drilling AJ, James C, et al. Efficacy of anti-biofilm gel, chitogel-mupirocin-budesonide in a sheep sinusitis model. *Australian Journal of Otolaryngology*. 2020;3.
431. Singhal D, Foreman A, Jervis-Bardy J, Wormald PJ. Staphylococcus aureus biofilms: Nemesis of endoscopic sinus surgery. *Laryngoscope*. 2011;121(7):1578-1583.
432. Psaltis AJ, Wormald PJ. Therapy of Sinonasal Microbiome in CRS: A Critical Approach. *Curr Allergy Asthma Rep*. 2017;17(9):59.
433. Wormald PJ. Outcomes after endoscopic sinus surgery-important and valuable to everyday practice. *Int Forum Allergy Rhinol*. 2012;2(6):435-436.
434. Bassiouni A, Chen PG, Wormald PJ. Mucosal remodeling and reversibility in chronic rhinosinusitis. *Curr Opin Allergy Clin Immunol*. 2013;13(1):4-12.
435. Bassiouni A, Naidoo Y, Wormald PJ. Does mucosal remodeling in chronic rhinosinusitis result in irreversible mucosal disease? *Laryngoscope*. 2012;122(1):225-229.
436. Jervis-Bardy J, Foreman A, Field J, Wormald PJ. Impaired mucosal healing and infection associated with Staphylococcus aureus after endoscopic sinus surgery. *Am J Rhinol Allergy*. 2009;23(5):549-552.
437. Cowin A, McIntosh D, Wormald P. Healing of wounds created in the nasal mucosa following endoscopic sinus surgery can be affected by different nasal packing materials. *Primary Intention: The Australian Journal of Wound Management*. 2002;10(3):114.
438. Weitzel EK, Wormald PJ. A scientific review of middle meatal packing/stents. *Am J Rhinol*. 2008;22(3):302-307.
439. Rajapaksa SP, Cowin A, Adams D, Wormald PJ. The effect of a hyaluronic acid-based nasal pack on mucosal healing in a sheep model of sinusitis. *Am J Rhinol*. 2005;19(6):572-576.
440. McIntosh D, Cowin A, Adams D, Wormald PJ. The effect of an expandable polyvinyl acetate (Merocel) pack on the healing of the nasal mucosa of sheep. *Am J Rhinol*. 2005;19(6):577-581.
441. Ha T, Valentine R, Moratti S, Hanton L, Robinson S, Wormald PJ. The efficacy of a novel budesonide chitosan gel on wound healing following endoscopic sinus surgery. *International Forum of Allergy & Rhinology*. 2018;8(3):435-443.

442. Venables WNR, B. D. . Modern Applied Statistics with S. Fourth Edition. , . In: Vol ISBN 0-387-95457-0. New York: Springer; 2002.
443. Harvey RJ, Psaltis A, Schlosser RJ, Witterick IJ. Current concepts in topical therapy for chronic sinonasal disease. *Journal of otolaryngology - head & neck surgery = Le Journal d'oto-rhino-laryngologie et de chirurgie cervico-faciale*. 2010;39(3):217-231.
444. Cabral JD, Roxburgh M, Shi Z, et al. Synthesis, physiochemical characterization, and biocompatibility of a chitosan/dextran-based hydrogel for postsurgical adhesion prevention. *Journal of materials science Materials in medicine*. 2014;25(12):2743-2756.
445. diZerega GS, Campeau JD. Peritoneal repair and post-surgical adhesion formation. *Hum Reprod Update*. 2001;7(6):547-555.
446. Carney MJ, Weissler JM, Fox JP, Tecce MG, Hsu JY, Fischer JP. Trends in open abdominal surgery in the United States-Observations from 9,950,759 discharges using the 2009-2013 National Inpatient Sample (NIS) datasets. *Am J Surg*. 2017;214(2):287-292.
447. Menzies D, Parker M, Hoare R, Knight A. Small bowel obstruction due to postoperative adhesions: treatment patterns and associated costs in 110 hospital admissions. *Ann R Coll Surg Engl*. 2001;83(1):40-46.
448. B BM, Lawson WE, Oury TD, Sisson TH, Raghavendran K, Hogaboam CM. Animal models of fibrotic lung disease. *Am J Respir Cell Mol Biol*. 2013;49(2):167-179.
449. Maxwell J. The production of pleural adhesions by kaolin injection. *Thorax*. 1954;9(1):10-13.
450. Delire B, Starkel P, Leclercq I. Animal Models for Fibrotic Liver Diseases: What We Have, What We Need, and What Is under Development. *J Clin Transl Hepatol*. 2015;3(1):53-66.
451. Vural M, Cosan TE, Uzuner K, Erkasap N, Cosan D, Bal C. Parenchymal Pressure Inconsistency in Different Brain Areas After Kaolin Injection into the Subarachnoid Space of Neonatal Rats. *Turk Neurosurg*. 2018;28(1):142-148.
452. Kraemer B, Wallwiener C, Rajab TK, Brochhausen C, Wallwiener M, Rothmund R. Standardised models for inducing experimental peritoneal adhesions in female rats. *Biomed Res Int*. 2014;2014:435056.
453. Ozel H, Avsar FM, Topaloglu S, Sahin M. Induction and assessment methods used in experimental adhesion studies. *Wound Repair Regen*. 2005;13(4):358-364.
454. <https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+630>.

455. Pairon JC, Jaurand MC, Kheuang L, Janson X, Brochard P, Bignon J. Sister chromatid exchanges in human lymphocytes treated with silica. *Br J Ind Med*. 1990;47(2):110-115.
456. Kondapalli SS, Czyz CN, Stacey AW, Cahill KV, Foster JA. Use of Kaolin-impregnated Gauze for Improvement of Intraoperative Hemostasis and Postoperative Wound Healing in Blepharoplasty. *J Clin Aesthet Dermatol*. 2016;9(6):51-55.
457. Choron RL, Hazelton JP, Hunter K, et al. Intra-abdominal packing with laparotomy pads and QuikClot during damage control laparotomy: A safety analysis. *Injury*. 2017;48(1):158-164.
458. Fallon JT. Specific Tissue Reaction to Phospholipids: A Suggested Explanation for the Similarity of the Lesions of Silicosis and Pulmonary Tuberculosis. *Can Med Assoc J*. 1937;36(3):223-228.
459. Potts RE. Kaolin granuloma. *The British journal of surgery*. 1953;41(167):295-299.
460. Sakari T, Christersson M, Karlbom U. Mechanisms of adhesive small bowel obstruction and outcome of surgery; a population-based study. *BMC surgery*. 2020;20:1-8.
461. Richter K, Ramezanpour M, Thomas N, Prestidge CA, Wormald PJ, Vreugde S. Mind "De GaPP": in vitro efficacy of deferiprone and gallium-protoporphyrin against *Staphylococcus aureus* biofilms. *Int Forum Allergy Rhinol*. 2016.
462. Mack D HM, Siemssen N, Laufs R. Association of biofilm production of coagulase-negative staphylococci with expression of a specific polysaccharide intercellular adhesin. *J Infect Dis* 1996; 174:881–883.
463. Vediappan RS, Bennett C, Bassiouni A, et al. A Novel Rat Model to Test Intra-Abdominal Anti-adhesive Therapy. *Frontiers in Surgery*. 2020;7(12).
464. Davey AKP, Maher PJMD. Surgical adhesions: A timely update, a great challenge for the future. *Journal of Minimally Invasive Gynecology, The*. 2007;14(1):15-22.
465. Ten Broek RPG, Stommel MWJ, Strik C, van Laarhoven C, Keus F, van Goor H. Benefits and harms of adhesion barriers for abdominal surgery: a systematic review and meta-analysis. *Lancet*. 2014;383(9911):48-59.
466. Trochsler M, Maddern GJ. Adhesion barriers for abdominal surgery: a sticky problem. *Lancet*. 2014;383(9911):8-10.
467. Cabral JD, McConnell MA, Fitzpatrick C, et al. Characterization of the in vivo host response to a bi-labeled chitosan-dextran based hydrogel for postsurgical adhesion prevention. *Journal of Biomedical Materials Research Part A*. 2015;103(8):2611-2620.

468. Vakalopoulos KA, Daams F, Wu Z, et al. Tissue adhesives in gastrointestinal anastomosis: a systematic review. *Journal of Surgical Research*. 2013;180(2):290-300.
469. Giles D, Talbot E. Suturing, Stapling, and Tissue Adhesion. In: Yeo CJMDF, ed. *Shackelford's Surgery of the Alimentary Tract*. 2019:1005-1013.
470. Olmarker K. Reduction of adhesion formation and promotion of wound healing after laminectomy by pharmacological inhibition of pro-inflammatory cytokines: an experimental study in the rat. *Eur Spine J*. 2010;19(12):2117-2121.
471. Lei W, Ehmsen RJ, Chiacchierini RP, Krelle JL, diZerega GS. Reduction of Leg Pain by Oxiplex Gel After Lumbar Discectomy in Patients With Predominant Leg Pain and Elevated Levels of Lower Back Pain: A Prospective, Randomized, Blinded, Multicenter Clinical Study. *J Spinal Disord Tech*. 2015;28(8):301-307.
472. Jordan J, Konstantinou K, O'Dowd J. Herniated lumbar disc. *BMJ Clin Evid*. 2011;2011.
473. Daniell JR, Osti OL. Failed Back Surgery Syndrome: A Review Article. *Asian spine journal*. 2018;12(2):372-379.
474. Fujii J, Kurahashi T, Konno T, Homma T, Iuchi Y. Oxidative stress as a potential causal factor for autoimmune hemolytic anemia and systemic lupus erythematosus. *World J Nephrol*. 2015;4(2):213-222.
475. [https://www.accessdata.fda.gov/cdrh\\_docs/pdf17/K172179.pdf](https://www.accessdata.fda.gov/cdrh_docs/pdf17/K172179.pdf).
476. Sheng H, Wang X, Kong N, et al. Neural interfaces by hydrogels. *Extreme Mechanics Letters*. 2019:100510.
477. Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. *Nat Rev Mater*. 2016;1(12):16071.
478. Richards PJ, Turner AS, Gisler SM, et al. Reduction in postlaminectomy epidural adhesions in sheep using a fibrin sealant-based medicated adhesion barrier. *Journal of biomedical materials research Part B, Applied biomaterials*. 2010;92(2):439-446.
479. Kim KD, Wang JC, Robertson DP, et al. Reduction of radiculopathy and pain with Oxiplex/SP gel after laminectomy, laminotomy, and discectomy: a pilot clinical study. *Spine (Phila Pa 1976)*. 2003;28(10):1080-1087; discussion 1087-1088.
480. [https://www.accessdata.fda.gov/drugsatfda\\_docs/label/2011/021825lbl.pdf](https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021825lbl.pdf).
481. Young RL, Cota J, Zund G, Mason BA, Wheeler JM. The use of an amniotic membrane graft to prevent postoperative adhesions\*\*Supported in part by a grant from the Clayton Foundation, Houston, Texas. *Fertility and Sterility*. 1991;55(3):624-628.

- 482. Power Coombs MR, Grant T, Greenshields AL, Arsenault DJ, Holbein BE, Hoskin DW. Inhibitory effect of iron withdrawal by chelation on the growth of human and murine mammary carcinoma and fibrosarcoma cells. *Experimental and Molecular Pathology*. 2015;99(2):262-270.
- 483. Kurahashi T, Fujii J. Roles of Antioxidative Enzymes in Wound Healing. *Journal of Developmental Biology*. 2015;3(2):57-70.
- 484. Gordon-Evans W. Dorsal Laminectomy. *Complications in Small Animal Surgery*. 2016.